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POLLEN DEVELOPMENT IN HYBRIDS OF *OENOTHERA LATA*×*O. LAMARCKIANA*, AND ITS RELATION TO MUTATION[1]

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY
LXXXIX

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(WITH PLATES II–IV)

This paper is a record, in part, of some investigations upon DEVRIES' mutants of Oenothera. The work was begun at the Marine Biological Laboratory, Woods Hole, Mass., in the summer of 1905; continued in the Botanical Laboratory of McGill University during the year 1905–6; and again at Woods Hole in the summer of 1906, where a larger garden of evening primroses was grown and studied, and material collected for further work; which is now being prosecuted at the Hull Botanical Laboratory, University of Chicago.

I have to thank Dr. B. M. DAVIS, under whose careful guidance the research was begun, for much attention and many suggestions and criticisms. I also have pleasure in acknowledging my indebtedness to Professor D. P. PENHALLOW, McGill University, for placing all the facilities of the Botanical Laboratory at my disposal, and for many kindly suggestions, to which Assistant Professor CARRIE M. DERICK also contributed, as the work progressed. The investigations at Woods Hole were carried on with the aid of scholarships from McGill University. I have to thank Dr. GEORGE T. MOORE for encouragement in the work of last summer, and also

[1] A preliminary paper was presented before Sections F and G of the American Association for Advancement of Science at New York, December 28, 1906.

Mr. GEO. M. GRAY for aid in planting and caring for the garden. These researches are being continued with the aid of a fellowship at the University of Chicago, and I am indebted to Professor JOHN M. COULTER and Dr. CHARLES J. CHAMBERLAIN for supervision.

Materials and methods

The first material of *Oenothera lata* was collected from a small garden of evening primroses at Woods Hole in 1905 from guarded seeds of DEVRIES. Stages were preserved for a study of sporogenesis and embryo sac development in *O. lata* and its hybrid forms; but the results recorded here will concern only pollen development, as the work on the megaspore and embryo sac has not been completed. The study of pollen development was taken up first because an interesting problem was presented by the abortion of the pollen, and because the reduction divisions in the microspore mother cell are for various reasons more easily investigated than those of the megaspore mother cell. The possibility of a cytological explanation of the segregation of characters in Mendelian hybrids, no one of which has yet been worked out on a chromosome basis, as well as the possibility of an explanation of the phenomena of mutation itself have also been kept in mind. The work has not yet reached a stage where it is possible to say that a cytological explanation for the Mendelian segregation of characters will be found, though this is possible. Some of the other results which will be described later, however, suggest that there may be found in the chromosomes a basis for the explanation of discontinuous variation or mutation, the suggestion being that the appearance of the mutant is preceded by certain morphological changes in the chromatin of the germ cells from which it originates, or, more exactly stated, that the germ cells from which a mutant arises differ in the structure of their idioplasm from the ordinary germ cells of the parent species.

Material has also been collected from *O. Lamarckiana* and several other of the mutants for comparison with *O. lata*.

The garden of 1906 at Woods Hole contained *O. Lamarckiana* and five of the mutants, all grown from guarded seeds of DEVRIES, which were planted in the greenhouse at the University of Chicago, the young seedlings being transplanted at Woods Hole about May 25.

By July 28, *O. rubrinervis* had begun blooming freely, and the other forms, including *O. lata*, began several days later. The earlier blooming of *O. rubrinervis* was very noticeable.

The history of the growth of these plants, which was observed with some care, will not be taken up here. Numerous crosses were made and other flowers self-pollinated for a study of the next generation, all necessary precautions being taken by means of bags, etc., to guard the flowers and to secure pure fertilization.

As stated by DEVRIES (**33**), the mutant *O. lata* does not mature its pollen and hence must be pollinated from another species, thus producing a hybrid in the second and succeeding generations. The seeds used were from a cross of *O. lata* with the pollen of *O. Lamarckiana*. These are said by DEVRIES (*l. c.* **1**:168) to segregate in the next generation, giving about 15–20 per cent. of *O. lata* plants and the remainder *O. Lamarckiana*, the characters of the latter thus being dominant.

My garden of 1906 contained fifteen plants from seeds of *O. lata* pollinated by *O. Lamarckiana*, ten of which conformed more or less completely to the characters of the pollen parent and four to the *lata* type, which is easily distinguished even in the rosette stage. One plant (no. 79), however, differed markedly from either of these forms, and was clearly a "mosaic" hybrid, *i. e.*, in some characters it resembled one parent and in some characters the other parent. The petals, however, were considerably larger than those of either parent species, and the sepals showed streaks of red, suggesting the sepals of *O. rubrinervis* but much paler. This character, however, is common to all the plants of *O. lata*×*O. Lamarckiana* having the *Lamarckiana* characters, and is occasionally seen to a less degree in *O. Lamarckiana* itself. The large ovaries and stout hypanthium, the greater pubescence on the young buds, and the broad leaves with their obtuse tips, are all characters of *O. lata*. But the leaves were scarcely at all crinkled (the more or less complete absence of crinkling being a character of *O. Lamarckiana* which distinguishes it easily from *O. lata*), and the general habit of branching and greater luxuriance of growth also correspond with *O. Lamarckiana*. This plant is mentioned as showing that segregation of the parental characters is not always complete in this cross, as this individual was fairly

intermediate in position between the two parents, though no fractionization of any characters was observed.

In killing material various fixing fluids were used, the most satisfactory being 1 per cent. chromacetic, a chromacetic solution made up as follows: 1 per cent. chromic acid, 70^{cc}; glacial acetic acid, 0.5^{cc}; water, 30^{cc}; and the following chromosmacetic formula: 1 per cent. chromic acid, 70^{cc}; glacial acetic acid, 0.5^{cc}; 2 per cent. osmic acid, 5^{cc}; water, 30^{cc}.

Sections were cut varying in thickness from 3 μ to 10 μ. The stains producing the best results were found to be Haidenhain's iron-alum-haematoxylin with orange G as a plasma stain, and Flemming's triple stain. The former is most satisfactory for the study of karyokinetic figures, particularly the chromosomes, but safranin and gentian violet give equally good results for synapsis stages.

Conditions of degeneration of pollen and tapetum in anther development

Unless otherwise stated, this account will refer to conditions observed in *O. lata*. The morphology of the *Lamarckiana* hybrid is very similar, so that the description would apply equally well to the latter, except where the pollen abortion in *O. lata* causes abnormalities.

Fig. 1 shows a transverse section of a single loculus in the "resting" pollen mother cell stage. The epidermal cells when normally developed have thick, highly cutinized walls at this early stage. Occasionally, when failure of anther development sets in at an earlier stage this cutinization does not take place, the cells remaining thin-walled. There are several "middle layers,"[2] generally about four. These are narrow radially and often show the effects of pressure from the large cells of the tapetum. The latter forms a well-organized layer of cells enclosing the sporogenous tissue. The granular appearance of the tapetal cells, owing to deeply-staining inclusions which fill the cytoplasm, easily distinguishes them from the middle layers. There are usually one to three longitudinal rows of pollen mother cells in a loculus, but the rows are not regular and a cross-section may show as many as five mother cells. In the great majority of cases the

[2] There is some variation in the use of this term. It is here applied to all the layers between the epidermis and the tapetum, because they are undifferentiated and all have the same developmental history.

tapetum is normal at this time, but occasionally some of the tapetal cells or even the mother cells may be found degenerating in this "resting" condition; in *fig. 3* both these conditions are present. Certain scattered tapetal cells show by their loss of turgor and their deeply staining contents that they are degenerating. Their nuclei may become flattened, but at first retain their nucleoli and nuclear wall. Later the latter breaks down and the nucleoli lose their outline and become indistinct, until finally the nucleus has disappeared. These changes are frequently accompanied by vacuolation. The cytoplasm stains progressively deeper with safranin and with haematoxylin. This may be due to the distributed condition of the chromatin, which diffuses through the cell after the nuclear membrane disappears, or to the formation of some acid product in the cytoplasm as the result of the mucilaginous degeneration. Similar staining reactions are shown by degenerating mother cells. *Fig. 4* shows a stage of this degeneration.

Most of the recent papers on pollen development in sterile hybrids do not describe the condition of the tapetal cells. GREGORY (**9**) mentions that the somatic cells surrounding the pollen mother cells in a hybrid race of *Lathyrus odoratus* are normal. JUEL (**13**), in his description of pollen development in the sterile hybrid *Syringa Rothomagensis*, makes no reference to the tapetal cells; nor does CANNON describe the condition of the tapetum in his two papers (**4, 5**). TISCHLER (**31**) finds conditions in the tapetal cells of hybrids of Ribes very similar to those described in some detail by BEER (**3**) in *Oenothera biennis* and *O. longiflora*, and which I have observed also in *O. lata* and *O. Lamarckiana*.

The tapetal cells in *O. lata* are uninucleate during the "resting" stage of the pollen mother cells. During the early presynaptic stages in the mother cells the tapetal nuclei divide mitotically, and the binucleate condition persists for some time. This first nuclear division in the tapetal cells may take place almost simultaneously throughout a loculus, so that many mitotic figures in various stages are often found in the same section. Later divisions are more irregular and are probably chiefly by amitosis, as stated by BEER (**3**) for *O. longiflora* and *O. biennis*, although occasionally two spindles may be found in the same tapetal cell.

My observations on the later stages of tapetal development agree in the main with those of BEER, and so will not be taken up here in detail. In some cases appearances suggest simultaneous division of a nucleus into several smaller nuclei, the latter often forming closely aggregated masses, as figured by BEER. At a still later stage, when the persistent pollen grains have begun to assume their adult shape, fusion of these masses of nuclei into one or two apparently occurs, and the tapetal cells return to a binucleate or even uninucleate condition. Both nuclei and cytoplasm now, however, show a very different appearance from the early uninucleate and binucleate condition, the nuclei being at this time much larger and having extremely little chromatic content. The cytoplasm, which is loosely reticular rather than granular at this time, frequently contains more or less indefinite and irregular chromatic masses. BEER describes the origin of these masses and compares them with the chromidial structures found by MEVES (**19**) in the tapetal cells of *Nymphaea alba*, and with similar bodies found by GOLDSCHMIDT (**8**) in the cytoplasm of certain animal tissues. These tapetal cells of the genus Oenothera offer excellent material for a further study of amitosis. It is interesting to note that the structure of the tapetal cells in the mutant *O. lata* is apparently identical with that of the well-known members of the genus, such as *O. biennis*. During these later nuclear divisions of the tapetal cells in *O. lata*, radial elongation of the cells may take place, but rarely enough to nearly fill the cavity of the loculus left by the degeneration (in such cases) of the mother cells. Transverse divisions of the tapetal cells are comparatively rare.

In the great majority of cases the tapetal cells begin to break down before disintegration in the mother cells sets in. As already stated, this disintegration may appear as early as the resting stage of the mother cells, where scattered cells begin to lose their turgor and degenerate. In later stages of development a great variety of conditions of disintegration of this tissue may be found. The tapetum may break down irregularly in different parts of a given loculus, or only on one side, or in patches. Different loculi of the same anther and different anthers of the same flower show wide variations in this matter.

POHL (**22**) states, followed by DEVRIES (*l.c.* **1**:292), that in *O.*

lata pollen development is normal up to the tetrad stage; that the tapetal cells later elongate and multiply, filling the whole loculus; and that the pollen cells in the mean time have broken down and disappeared, only a few small scattered grains remaining. The first statement is erroneous, because degeneration may take place in the pollen mother cells before the reduction divisions (*figs. 3, 4*). POHL'S account of the history of the tapetal cells is also contrary to my observations. It is true in general that the tapetal cells continue active during synapsis and the reduction divisions in the pollen mother cells, although breaking down frequently begins at this time, or even earlier, in the "resting" stage, as already mentioned. But in later stages, when the pollen cells have largely disappeared after the reduction divisions, leaving an empty cavity, the normal condition is not as POHL states—ingrowth of the tapetal cells to fill the cavity—but partial or complete disappearance of the tapetum; and in the latter case ***subsequent ingrowth*** of the middle layer cells behind to fill more or less completely the cavity (*figs. 5, 6*). Stages of this process are found in which the disintegrating tapetal cells form a thin layer lining the locular cavity (which is usually empty under these conditions), the remains of this layer sometimes appearing later as a thin, homogeneous, deeply staining ring (*fig. 5*), which may afterwards completely disappear. The middle layer cells, which now line the cavity, in the meantime become rounded on their inner surfaces, owing to the release of the pressure of the tapetal cells. They then push in to fill the cavity and may even multiply by mitotic division. The cavity of the loculus may thus become completely closed (*fig. 6*).

POHL'S error in mistaking these middle layer cells for tapetum was probably due to a hasty and insufficient examination, for in carefully prepared sections the former have a totally different appearance from the tapetal cells, for which they could not possibly be mistaken. While the latter are easily recognizable by their densely granular contents and conspicuous nuclei, as well as by their rectangular shape, the middle layer cells have no granular contents, are uninucleate, with small nuclei having little chromatin, and are rounded and irregular in shape. POHL'S error is probably to be attributed to his failure to observe the degeneration and disappearance of the tapetum before the ingrowth of the middle layer cells takes place, as well as to distin-

guish between the cells of the middle layers and the tapetum by their appearance.

This condition, where the tapetum breaks down early and the middle layer cells then grow in and fill up the loculus, is common and may occur in one loculus of an anther when the adjoining loculi show normal functioning tapetum. The ingrowth is frequently irregular, occurring more rapidly on one side of the cavity, thus causing the latter to lie excentrically in the anther lobe and to assume a narrow and flattened or irregular shape. This ingrowth of middle layer cells does not initiate the disintegration by pressure on the tapetal cells, or in any other way, for it only begins after the tapetum has more or less completely disappeared, which is long after the first appearance of degeneration in the tapetal cells. That the tapetal cells are necessary for the nourishment and growth of the pollen grains is shown by the entire absence of the latter in such cases long before the cavity is obliterated.

Another and perhaps more common condition is for the tapetum to persist through the tetrad divisions and until the persistent pollen grains attain a large size. The loculus meantime enlarges considerably by growth (*fig.* 7), and also by the flattening and breaking down of the middle layers, as would be expected in normal pollen development. The tapetal cells also generally become flattened at this time, and stretched out so as to line the larger cavity created partly by growth and partly by the degeneration of the middle layers. In such loculi pollen grains in various stages of development are frequently though not always found, although when present they almost invariably assume irregular and bizarre shapes, though they may contain nuclei which are normal in appearance. Rarely pollen grains may be seen which have the triangular disk-shaped appearance characteristic of the pollen in this genus; but such grains have never been observed to reach full size, although abnormally shaped grains may do so sometimes.

It is evident that the break-down of the middle layers, as mentioned in the last paragraph, is due to pressure from the functioning tapetum, for the cells of the middle layer become flattened radially and lose their contents, while the tangential walls may persist for some time, forming irregular concentric rings of dark-staining substance. Later

these completely disappear, leaving no trace of the middle layers. The tapetum then lies directly against the epidermis.

The presence of pollen grains in various stages of development when the tapetum persists, and their complete absence when the tapetum disappears early, shows that the failure of the tapetum to develop is at least one factor in the abortion of the pollen. POHL'S conclusion that "die Zellvermehrung und relative Persistenz der Tapetumzellen hemmt die Pollenentwicklung" is evidently an error, for, in the great majority of cases at least, where the tapetum persists, it does not show abnormal radial elongation or growth to fill the locular cavity, the tapetal cells in older stages becoming instead very much flattened radially, as already seen. The cases where the tapetum does not persist, but where the cells of the middle layers grow in and obliterate the cavity, have already been described. In short, when the tapetum persists the middle layers break down, as is the usual condition in normal pollen development; but when the tapetum degenerates at an abnormally early stage, the cells of the middle layers grow in and fill the loculus.

History of the pollen mother cells

PROPHASE AND SYNAPSIS

The description thus far has dealt chiefly with the general morphology of pollen development in *O. lata;* and the development of the pollen mother cells will now be taken up in greater detail. It is a noteworthy fact that the mother cells of a given loculus show but little variation in their stage of development. Generally, however, there is a slight progression from top to bottom of a loculus; *e. g.*, when the pollen mother cells at the top of a loculus are in pre-synaptic stages, those at the bottom may be in the late post-synaptic stages of the prophase; or when the mother cells at the top of a loculus are in metaphase of the heterotypic division, those at the bottom may have reached the telophase with reconstructed nuclei. In comparing different loculi of an anther or different anthers of the same flower, the variation is often much greater. Thus in some loculi the pollen mother cells may be in synapsis; while in others of the same or another anther in the same flower, they may be in metaphase of the heterotypic

division. This irregularity is probably connected with the failure in pollen development.

The cytoplasm of the mother cells in the "resting" condition contains numerous large spherical starch grains. In safranin-gentian they stain pale blue and the central hilum is made more prominent by its retaining a highly refractive globule of xylol. These starch grains are thus very conspicuous objects in the mother cells (*fig. 3*). With iodine they appear brown and angular, and the iron-haematoxylin stain leaves them invisible, so that very different appearances of the mother cells are obtained according to the method of treatment. During the prophases of the first mitosis the starch grains become more abundant, until the cytoplasm is literally packed with them. This reserve store of nutriment is used up during the reduction divisions, and no starch grains are to be seen in the pollen tetrads. In iron-haematoxylin the cytoplasm of the pollen mother cells frequently presents a rather obscurely radiate appearance during the pre-synaptic stages. In the earlier or "resting" condition of the nuclei in the mother cells they contain one large nucleolus, which is commonly placed near the nuclear wall and contains one to several vacuoles of varying size with highly refractive walls (*fig. 9*). In addition, two or more smaller chromatic bodies are usually found distributed near the nuclear membrane. They appear connected and often surrounded by the delicate reticulum scattered through the nucleus. The large nucleolus is several times the size of the smaller bodies. Sometimes it is in the center of the nucleus, and the smaller bodies are appressed to the nuclear wall. Vacuoles are usually observed only in the large nucleoli, but occasionally minute ones may be seen in the smaller chromatic bodies. More rarely two large nucleoli of nearly equal size may be found. The small round chromatic masses arise from the more angular chromatic bodies found at the nodes of the reticulum in an earlier stage (*fig. 8*). These bodies probably correspond to OVERTON'S "prochromosomes," though they have not been studied fully. The next stage leading towards the synapsis condition is apparently the beginning of contraction of the nuclear reticulum. The nuclear membrane is often extremely delicate at this time, and as indicated in *fig. 10*, the nucleolus is frequently surrounded by a compact reticulum which has contracted from the nuclear membrane,

and in which are embedded, especially near the margin, the "prochromosomes," forming an incomplete circle around the periphery. The material in which are found the beginnings of this contraction, as well as its later stages, was fixed and stained in the same manner as the slightly earlier stages in which it does not occur.

The transition from this stage to the regular spirem stages has not been followed. The pre-synaptic stages with a continuous spirem last for a comparatively long time, as shown by the frequency of their occurrence. The spirem is at first extremely delicate, very long and tangled (*fig. 11*). One large nucleolus is always seen at this time, but one or more smaller ones may also be present. In a later stage the nucleolus is for a time closely appressed and somewhat flattened against the nuclear wall. Loops of the spirem extending to the wall frequently give a false appearance of doubling (*fig. 14*), but though a great many spirem stages have been studied, a double thread has never been observed. The small size of the nuclei, however, and the delicacy of the spirem thread make it very difficult to determine this point.

The spirem gradually contracts into a dense ball with a few loose threads projecting irregularly (*fig. 12*). In this closely contracted condition it may form a body about the size of the nucleolus, which can only be distinguished from the latter by its somewhat irregular outline (*fig. 13*). In the next stage observed the spirem is again loosely arranged in the nuclear cavity, but is greatly contracted in length and several times the thickness of the original spirem before the contraction stage. This is shortly before the spirem breaks up into chromosomes, and the thread is at this time about the same thickness as the chromosomes in the later prophase. Throughout the earlier stages of the prophase the spirem thread is continuous, and, judging from its frequency, the duration of the long delicate spirem previous to the synapsis stage is considerably longer than that of the short thick spirem after synapsis and before it segments into chromosomes. In the latter stage, before segmentation into chromosomes, there is frequently found, besides the spirem, a ring-shaped body of chromatic material exactly like the spirem in thickness and staining power. This has evidently been cut off from the spirem (*figs. 17, 18, 19*). Frequently there are two such rings.

Fig. 16 probably shows the origin of one of them by the cutting off of a loop of the spirem. There can be no doubt that these bodies arise from the spirem thread previous to the breaking up of the remainder of the thread into chromosomes. The nucleoli at this time are large, globular, and deeply-staining. *Fig. 20* shows a slightly later stage, in the prophase, where two of these ring bodies, which I shall call "heterochromosomes"[3] on account of their later history, are present in addition to the nucleolus and several chromosomes. That these are not the ring-shaped chromosomes frequently found in the prophase of the heterotypic mitosis is shown by their later history; for the rings do not segment in metaphase, forming V- or U-shaped structures, but pass towards the poles of the spindle or into the cytoplasm undivided and retaining their ring form. The shape of the ordinary chromosomes, which are nearly globular or slightly elongated and pear-shaped, precludes the possibility of the pairs forming the X, Y, V, and O shapes which characterize the heterotypic mitosis when the chromosomes are long and rod-shaped.

The heterochromosomes are not always formed, however, and hence if the original pollen mother cells were all alike in the early synapsis stages, two kinds of pollen cells must result; for, as will be shown later, the heterochromosomes probably represent a portion of the chromatin which is rejected and disappears in the cytoplasm, so that the daughter nuclei must differ in their chromatin content. *Fig. 21* shows thirteen chromosomes in the prophase at a later stage, when they are condensed to a considerably smaller size. The adjacent section shows a nucleolus and one chromosome, but no heterochromosomes are present. It has been determined from a number of counts in the prophase that the sporophyte number of chromosomes, exclusive of the heterochromosomes (or at least when the latter are not present), is fourteen. Whether the number is the same when the heterochromosomes are present has not yet been determined with

[3] The term was proposed by MONTGOMERY as a general term for the aberrant chromosomes in Hemiptera, and is used in preference to "idiochromosomes," because the latter term has been applied to a certain type of heterochromosomes by WILSON (37). More recently MONTGOMERY (20) has proposed the term "allosome" as a general term for all aberrant chromosomes, and "monosome" for one which passes undivided to one of the daughter nuclei. The latter term would be most suitably applicable to the bodies described here.

certainty, but it appears probable that it is less. BEER (3) states that the reduced number of chromosomes in *O. longiflora* is seven; hence these two species probably agree in the number of their chromosomes. Frequently the chromosomes in the late prophase show evident pairing, but it is usually difficult to represent this accurately in a drawing, because the members of a pair generally lie in different planes. However, indications of such a pairing are shown in *figs. 21* and *22*. The positions of the chromosomes make it certain that this is an apposition and pairing of whole chromosomes and not a splitting. This is evident, for the chromosomes are completely separated when first seen in prophase and are only arranged in pairs later, on the equatorial plate.

Fig. 22 shows another prophase with ten chromosomes, no heterochromosomes, and a nucleolus in which the stain has been withdrawn from its outer shell except on one side. This condition of the nucleolus is common enough to cause one to suspect that it possesses some significance. Possibly the side of the nucleolus in which the amphipyrenin retains the stain is the one which was earlier attached to the nuclear wall. However I have no observations to prove this. The nucleolus now rapidly loses its staining power, and in the late prophase, after the chromosomes are formed, frequently stains only with orange G in an iron-haematoxylin-orange stain. It gives no indications of loss of shape or size before it disappears, and I think the most probable supposition is that it is quickly dissolved in the cytoplasm when the nuclear membrane breaks down. The evidence is clear that it disappears about this time, and it is very rarely seen as late as metaphase. I may state here that the stages of its reappearance in the reconstructed daughter nuclei at the end of the heterotypic mitosis are in the reverse order of its disappearance. Soon after the nuclear walls appear around the daughter nuclei, when the nuclear sap has increased in quantity, the nucleoli appear (*fig. 42*) as pale yellow-staining bodies, usually several in number and frequently attached to the nuclear wall. It is impossible to believe that these bodies are reconstituted from fragments of the original nucleoli thrown out into the cytoplasm during the first mitosis and collected again into the daughter nuclei, as described by SCHAFFNER (26) for the megaspore divisions in *Lilium philadelphicum*. For

in the last stage of their disappearance they retain their shape but lose their staining power, as if the deeply staining substance (chromatin?) had been dissolved away, leaving the yellow-staining ground-substance, and in the first stage of their reappearance the yellow-staining material only is present. I think this precludes the possibility of dentifying the bodies in the cytoplasm, which stain like chromatin, as fragments of the nucleolus.

THE HETEROTYPIC MITOSIS

Thus far the description of events in the pollen mother cell has related only to *O. lata*, because the corresponding stages in the *O. Lamarckiana* side of the hybrid have not been studied. There is every reason to believe, however, as will be shown later, that the history is the same except for the difference in the number of chromosomes, because heterochromosomes are found very commonly in the *O. Lamarckiana* side of the cross, in the metaphase of the heterotypic mitosis, and they doubtless have the same origin as in *O. lata*. These heterochromosomes are the first described in plants, and a discussion of their possible significance will be deferred to the end of the paper.

Fig. 23 shows a metaphase of the heterotypic mitosis in *O. lata*. There are shown twelve chromosomes (some of which are in pairs of equal size) and a heterochromosome. *Fig. 24* shows two heterochromosomes of unequal size far out in the cytoplasm.

Figs. 25 and *26* are from sections of spindles showing the heterotypic mitosis in the *O. Lamarckiana* hybrid. In *fig. 25* the heterochromosomes are of equal size and are found on the same end of the spindle. This condition is very commonly found. In *fig. 26* the heterochromosomes are in the cytoplasm and not attached to the spindle; one of them is larger than the other and they are apparently proceeding towards opposite poles of the spindle.

Fig. 27 is a side view of a spindle showing a small chromosome out of position near the equatorial plate. A similar body is seen in *fig. 26*. What significance these may have is not known. Their early history has not been observed. They are not ring-shaped, like the typical heterochromosomes, but they frequently stain less deeply than the regular chromosomes. These bodies were only

observed a few times, while the heterochromosomes are very common although they are certainly absent from some pollen mother cells.

Figs. 29, 30, 31, and *32* are sections of the equatorial plate of the *O. Lamarckiana* hybrid, showing one or more heterochromosomes. The chromosomes are so densely grouped at this time that it is often impossible to make out the individuals. This renders some of the figures unsightly, but they are intended to be merely accurate representations of what is seen on the spindle.

After passing into the cytoplasm, where they are generally found in metaphase, the heterochromosomes apparently degenerate and disappear. They gradually become smaller and stain less deeply, though retaining their ring form, as in *fig. 32*. Finally they are so small and inconspicuous as to be scarcely distinguishable from the granules (microsomes) of the cytoplasm. It is probable that they usually disappear before the telophase of the heterotypic mitosis. Several other points in regard to their history have yet to be determined.

Figs. 34 and *35* are equatorial views. *Fig. 34* shows several chromosomes together with a heterochromosome, and also a very large or giant ring chromosome, which apparently contains enough chromatin for several chromosomes. *Fig. 35* shows another; and these two cases were the only ones observed. They are apparently quite distinct from the heterochromosomes already described, although they are ring-shaped. No other observations have been made in regard to them.

Fig. 35 is a polar view of the equatorial plate of the heterotypic mitosis in the *O. Lamarckiana* hybrid. The apparently unequal pairs are probably due to foreshortening, owing to one chromosome lying in a lower plane. *Figs. 36* and *37* are early anaphases in the *O. Lamarckiana* hybrid. *Figs. 38* and *39* are late anaphases. *Fig. 38* shows clearly the premature splitting of the chromosomes for the homotypic division. This splitting may occasionally be observed in late metaphase, and in the late anaphase the separation of the halves is often complete. V-shaped chromosomes were not observed, the split apparently taking place simultaneously throughout. Thus it is clear that the heterotypic mitosis distributes whole chromosomes, which were arranged in pairs (though not at first in contact) in the prophase; while the homotypic mitosis is a typical division, separating

the halves of the chromosomes already formed by splitting in the anaphases of the heterotypic division. The heterotypic is thus the reducing division. The shape of the chromosomes of course makes it impossible to determine whether the second splitting is longitudinal or transverse.

Fig. 40 shows a telophase in which the reconstruction of the daughter nuclei has begun. Delicate nuclear walls are appearing and the chromosomes have assumed angular, lobed, and irregular forms, but very little nuclear sap has yet appeared. Materials staining like chromatin are scattered through the cytoplasm. *Fig. 41* shows the same stage, in which bodies as large as chromosomes and taking the same stain are scattered through the cytoplasm (particularly in the region of the daughter nucleus). It has already been shown, I think, that these bodies cannot be fragments of the nucleolus. Neither are they likely to be fat bodies, since the cytoplasm in earlier stages contained abundant storage nutritive materials in the form of starch. It seems then most reasonable to conclude that these bodies are actually chromosomes, and fragments of chromosomes which for some reason have become separated from their fellows on their way to the poles of the spindle. It is difficult to understand, however, what forces carry them into the cytoplasm, for among hundreds of spindles studied no irregularities in their structure have been observed which would cause the chromosomes to be distributed irregularly. Indeed the heterotypic spindle is notably regular in form. The earlier stages of spindle formation have not been studied, however, and it may be that these would account for some of the chromosomes not being on the spindle. It is doubtful if the heterochromosomes are ever attached to the spindle. Of course the position of chromosomes near the nuclear membrane is easily accounted for by their having merely failed to pass to the poles of the spindle, but this will not explain the position of many which are found scattered in the cytoplasm throughout the cell and frequently near its periphery.

Fig. 42 is a polar view of a daughter nucleus in a later stage of reconstruction. The nucleus has grown greatly in size, owing to a very rapid increase in the nuclear sap. At the same time the chromosomes have become drawn out into irregular shapes, are much reduced in size and stain less deeply. Several nucleoli have appeared, but

they do not take the chromatin stain. In an iron-haematoxylin-orange stain they take only the orange; thus it is difficult to see how the view that the nucleoli of the daughter nuclei are reconstructed from fragments of the former nucleolus (SCHAFFNER **26**) can apply here, for fragmentation of the nucleoli before their disappearance has never been observed, and their disappearance is preceded by loss of staining power, as if the chromatic-staining substance were first dissolved away, leaving the pale ground-substance. The method of final disappearance of the latter has not been observed. As already mentioned, the reverse series of events occurs in the reappearance of the nucleoli in the daughter nuclei. When first observed in the daughter nuclei, they have their characteristic size and shape, but stain yellow and only acquire the chromatic stain later. They were never observed until there was a considerable accumulation of nuclear sap, and are certainly not visible until some time after the membranes of the daughter nuclei have been formed. These changes in the staining power of the nucleoli might be attributed to chemical changes in the nuclear sap, the medium in which they float, such as would alter the staining reaction of bodies contained in it. But while the nucleolus undergoes these changes before its disappearance, the chromosomes remain constant in their staining properties, which makes it appear more probable that the change in staining is due to actual loss of material from the nucleolus.

Several of the figures (*figs. 37, 38, 39*) show that the number of chromosomes in the *O. Lamarckiana* hybrid is greater than in *O. lata*. A count has not been made in the prophase, but from numerous counts in the metaphases and anaphases, the sporophyte number is found to be at least twenty and probably very near that number (*fig. 37*). It will be remembered that the sporophyte number in *O. lata* is fourteen. The bearing of these facts will be discussed later.

The study of several other interesting features of the telophase of the heterotypic as well as of the homotypic division has not yet been completed.

The pollen tetrads

The pollen tetrads in *O. lata* show numerous interesting features. As already stated, development to this stage frequently takes place,

though not always. When development takes place, the tapetum is also found to be normally developed and functional. In some cases, as in *figs. 43* and *44*, the normal number of four nuclei is found (three shown in the figures), though these may vary in size (*fig. 43*) and in chromatin content. In other cases (*fig. 45*) chromatic bodies are found in the cytoplasm. In *fig. 46* one of the nuclei has two others lying near it. *Fig. 47* shows a tetrad with extra nuclei. Occasionally large and irregular shaped nuclei are found, as in *fig. 51*. *Fig. 48* is a section of a tetrad showing an extra nucleus formed from a single chromosome and connected by spindle fibers with one of the larger nuclei. *Fig. 49* is a portion of a tetrad showing a nucleus with a smaller one beside it; and *fig. 50* similarly shows two small extra nuclei, each containing two chromosomes. Pairing of the chromosomes in the large nuclei of the tetrad is sometimes seen. Chromosomes are frequently found just outside the nuclear wall; this is probably due to tardiness in passing to the poles of the spindle. Such chromosomes may or may not be surrounded by a "nuclear membrane." Most of the pollen tetrads show evidences of breaking down, and a great many are more or less completely degenerated. In the *O. Lamarckiana* hybrid irregular-shaped pollen grains are quite common in some anthers; *fig. 52* shows some of these irregularities. There may be extra lobes or fewer lobes than the normal, and they may be irregularly placed. Occasionally complete sterility of certain anthers of a flower may be found in the *O. Lamarckiana* hybrid, as in *O. lata*.

Discussion

A full discussion of the literature bearing on the facts set forth in this paper will not be attempted at this time, as the work touches upon several distinct fields. But I shall mention a few of the most important papers bearing directly upon these investigations, and close with a short discussion of the general bearing of the most important results.

THE EXTRA NUCLEI IN POLLEN TETRADS

WILLE published a paper (**34**) on pollen development in the angiosperms in 1886, before botany had acquired its present technique, in which he cites a list of cases of extra nuclei in the tetrad

divisions. He remarks that these are chiefly cultivated plants, and suggests that the extra nuclei are indications of greater variability in structure resulting from cultivation. This was of course before the modern views of chromosome reduction and alteration of generations had been developed. The fact that extra nuclei are so commonly found among the irregularities in the divisions of the pollen mother cells in hybrids, together with the well-known fact that cultivated plants are very commonly hybridized, would seem to lay open to suspicion the purity of any species in which such irregularities occur. Even *Hemerocallis fulva*, which is commonly quoted as a pure species having these irregularities, is well known to have been long under cultivation, and three varities of it are described by BAILEY (**2**). The other members of the list given by WILLE (*l. c.* 60), with mention of their cultivation and varieties, taken from BAILEY'S *Cyclopedia of American Horticulture*, are given below.

Name	Varieties	
Ampelopsis hederacea =A. quinquefolia	..	cult. climbing vine
Azalea indica	..	cult. and hybridized
Begonia sp	..	cult. and variable
Charitzania ilicifolia	..	not in Bailey
Cornus sanguineus	2	cult. shrubs
Elatine hexandra	..	not in Bailey
Ficaria ranunculoides	..	not in Bailey
Fuchsia sp	..	cult., very variable, and probably hybridized
Funkia ovata	1[4]	cult.
Hemerocallis fulva	3	cult.
Lonicera coerulea	..	cult. shrubs
Prunus cerasus	..	cult. and hybridized
Rumex patientia	..	cult. and nat.
Scleranthus annuus	..	not in Bailey
Stellaria glauca	..	not in Bailey
Symphytum officinale	1	cult.
Syringa persica	2	cult.

An examination of the list shows that all but five are forms cultivated in America, and most of these are either open to suspicion as hybrids or known to have been hybridized. In the light of these facts, a careful examination of the history of all forms in which such extra nuclei are known to occur would be advantageous. It would

[4] Two other varieties are given by J. G. BAKER in "A revision of the species of herbaceous gamophyllous Liliaceae" in Jour. Linn. Soc. 11:366. 1871.

thus be possible after a time to decide whether they result from hybridization only, or whether they may be the result of some other constitutional derangement caused by cultivation. It should be mentioned in this connection that Miss LYON (**16**) described five and six spores in a mother cell of *Euphorbia corollata*, which is a common species in this region. In this case the daughter cells are stated to be all of the same size, however, which suggests that the number may have been produced by extra divisions of one or more of the daughter cells, rather than by some of the chromosomes becoming scattered in the cytoplasm. The same explanation may apply to some of the other forms mentioned; and it is also necessary to assume extra divisions in cases such as *Lonicera coerulea*, where as many as fourteen daughter cells are figured in one mother cell. FULLMER (**7**) thought that such extra mitoses would account for most of the conditions in Hemerocallis, but in this he was probably incorrect.

A more careful study of some of these forms, and of some of those said to produce only one, two, or three pollen grains from a mother cell, as described in the paper of JUEL (**14**) on Carex, would doubtless furnish many interesting results and aid in a determination of the causes of these irregularities.

THE PHENOMENA OF POLLEN DEGENERATION

During the last few years a number of cases of sterility in the pollen of hybrid plants have been described, with interesting morphological and cytological results. JUEL (**13**) in 1900 described pollen development in the sterile *Syringa Rothomagensis*, which is a cross between *S. vulgaris* and *S. persica*. He also studied the corresponding stages in the parents for comparison. In *S. vulgaris* only about 50 per cent. of the pollen grains were found to be normal, and in *S. persica* good grains were few. Practically the same conditions of development were found in *S. persica* as in the hybrid. A great variety of irregularities in the reduction divisions was found in the hybrid. As in *O. lata*, the degeneration sometimes began as early as the synapsis stage, but generally the tetrad divisions were completed. A drawing apart of the nucleus into two may occur in the spirem stage or after the spirem has segmented into chromosomes. The latter at least could not be called amitosis. In the first reduction

mitosis chromatin appears in the cytoplasm, as in the Oenothera hybrids studied. The spindle is also sometimes multipolar and irregular. Several nuclei may be found in a tetrad, and also chromatin scattered in the cytoplasm, as in *O. lata*. This hybrid shows a greater variety of irregularities than have been observed in any other hybrid described.

GUYER (**10, 11**) found in the spermatogenesis of hybrid pigeons similar conditions with extra and irregular spindles and incomplete pairing of the chromosomes. CANNON (**5**) found in hybrid cotton, *Gossypium barbadense* × *G. herbaceum*, that degeneration of the pollen mother cells sometimes begins in the resting stage previous to synapsis. The divisions in some of the pollen mother cells are amitotic, the nucleolus dividing first, followed by equal or unequal segmentation of the nucleus and its reticulum. In the case of equal segmentation, pollen tetrads of normal appearance may be formed. Multipolar spindles, with many small nuclei, were also found. In fertile hybrids of certain races of peas, which obey Mendel's law, CANNON (**4**) found no difference in pollen development between the hybrids and their parents. He states that there is a pairing of the chromosomes in the telophase of the last sporogenous division previous to synapsis. It should be said, however, that these two papers are not as complete and satisfactory as some of the others.

ROSENBERG'S (**23, 24**) study of pollen development in *Drosera longifolia obovata*, a hybrid of the intermediate type, between *D. rotundifolia* and *D. longifolia*, but nearer *D. longifolia*, shows that some of the smaller chromosomes of *D. longifolia* are left behind in the cytoplasm in the first and second divisions, where they also form small nuclei. Later the greater number of the pollen grains lose their contents. The embryo sac development also usually stops at the binucleate stage. The sterile hybrid sweet peas (*Lathyrus odoratus*) studied by GREGORY (**9**) show an earlier degeneration of pollen than the other cases cited. Portions of the spirem are often destitute of chromatin and the chromosomes frequently are pale, which GREGORY attributes to lack of nutrition. The nucleolus, he thinks, serves to store most of the chromatin between the reduction mitoses. Other conditions simulating amitosis in the pollen mother cells are illustrated. Degeneration of the pollen mother cells usually takes place

in the prophase, but occasionally the first spindle is formed, though it is irregular. Development never proceeds to a later stage. The sterility is here a Mendelian character, recessive and correlated with a certain somatic character, the two always appearing together.

TISCHLER (**31**) ascribes the sterility of the hybrids of Ribes and Bryonia, as well as those of *Lathyrus odoratus*, to the influence of long culture. He studied three hybrids of Ribes: (1) *R. intermedium* = *R. sanguineum* × *R. nigrum;* this hybrid is fertile, producing 10–15 per cent. good pollen grains; (2) *R. Gordonianum* = *R. aureum* × *R. sanguineum;* absolutely sterile and showing great vegetative luxuriance; (3) *R. Schneideri* = *R. grossularia* × *R. nigrum;* sterile. He finds a splitting of the spirem in the strepsinema stage, and that the chromosomes when formed lie in pairs in the prophase. As in *O. lata*, the heterotypic is a reduction division, the homotypic an ordinary equation division, and the chromosomes split (longitudinally) in the anaphase of the first mitosis. In all the hybrids, as well as in the parents, *e. g., R. sanguineum*, the reduction divisions are mostly normal, though in *R. Gordonianum* occasional extra spindles are found in the cytoplasm; otherwise its tetrad divisions cannot be distinguished from those of *R. intermedium;* but the sterile grains do not grow in size. The first evidences of degeneration appear then in Ribes later than in any of the others mentioned, *i. e.*, usually after the reduction divisions are completed.

In a sterile hybrid of *Begonia alba* ♀ × *B. dioica* ♂, TISCHLER (**32**) finds that when the reduction divisions in the pollen mother cells are irregular, they are invariably preceded by abnormalities in synapsis; but that when synapsis is accomplished regularly the reduction divisions are also carried through without irregularities. There is often unequal division of the chromosomes on the heterotypic spindle. In the tetrad divisions, chromosomes in the cytoplasm and extra nuclei are relatively seldom found. The pollen grains are very often degenerate and of unequal size. In the female gametophyte the megaspore mother cells divide normally, but the embryo-sac mother cell degenerates. Whether another embryo-sac is formed later from the sporophyte tissue, as shown by TISCHLER for *Cytisus Adami* (**29**), *Ribes Gordonianum*, and *Syringa chinensis* (**30**), has not been determined.

In some of the papers sufficient attention has not been given to a comparison of the adult characters of the hybrid with those of the parents, *i. e.*, whether the hybrid is a mosaic, Mendelian, an intermediate, or goneoclinic to one parent, constant and invariable or breaking up into different types, etc. It would be particularly valuable to know in every case to what particular type the hybrid whose germ cells are studied conforms, and what variations if any occur.

THE POSSIBLE CAUSES OF STERILITY IN HYBRIDS

That hybrids are frequently sterile has of course long been known and commented upon. The recent morphological and cytological papers already cited have not reached an explanation of its cause, though various suggestions have been made. Some of the causes suggested may be classified into several general categories which are not, however, necessarily mutually exclusive: (1) lack of "nutrition" in the parts affected, causing, *e. g.*, failure in development of the tapetum or low staining capacity of the chromatin in the germ cells; (2) the influence of long culture (whatever that may be); (3) irregularities of development in the germ cells, appearing during synapsis or the reduction divisions and due to some lack of harmony (variously expressed) between the idioplasms; (4) some more deep-seated phenomenon affecting the physiology of the whole plant. This is not an attempt to classify all the possible causes of sterility suggested, but it will be seen that the factors are stated from widely differing points of view and with varying amounts of definiteness. Some of them might be viewed as effects rather than as causes.

In *O. lata*, if the tapetal cells always showed a degeneration before the pollen mother cells, we might conclude that the failure of the tapetum to function (in the secretion of nutriment for the pollen grains, which is generally conceded to be its chief function) was the immediate cause of the abortion of the pollen. But this is not always the case; for degeneration in the pollen mother cells may appear as early as in the tapetal cells or earlier; while GREGORY states that in the hybrids of *Lathyrus odoratus* the "somatic cells" are normal throughout. GUYER (**10**) suggested in 1900 that incompatibility of the chromosomes or plasms, as shown in the prophases of the first reduction division, is the source of the subsequent irregularities in

spermatogenesis, and consequently the cause of sterility in hybrid pigeons. But in the case of *O. lata* such an explanation can scarcely suffice, for we cannot imagine that any incompatibility or failure of fusion of the maternal and paternal plasms or chromosomes could almost simultaneously bring about the disintegration of the tapetal cells. GREGORY (**9**) suggests that "it seems more likely that sterility of the male organs is the expression of some deeper-lying phenomenon affecting the whole plant," and a similar view is held by TISCHLER (**31**). Thus it appears that the cause must be sought in some more wide-spread phenomenon in the hybrid, causing general lack of nutrition of the parts which degenerate; hence the tapetum and pollen mother cells degenerate simultaneously and for the same reason. Recent papers, which need not be referred to in this connection, have also shown that hybridism may cause abnormalities or failure of development in the female gametophyte, though this is apparently less common. These facts, together with the usually normal and in some cases increased luxuriance of the sporophyte, point to the gametophyte as that part of the life history which suffers chiefly from "lack of nutrition." The sporophyte generation can evidently be carried through successfully if fertilization is once accomplished; but something which first displays itself as lack of nutrition (as shown by the failure of the tapetum to function, as well as perhaps by the pale staining of the chromatin of the pollen mother cells in some cases) prevents the normal development of the gametophyte.

That this is not the whole case, however, is shown by the fact that the reduction divisions are abnormal, even when the tapetum appears perfectly developed and functional. Hence something other than "lack of nutrition" must be the cause of these abnormalities in development. The ingrowth of the median wall layers to fill the loculus after the disappearance of the pollen, points rather in the opposite direction. But perhaps a harmony of these two points of view may be reached. If the view, recently emphasized by COOK and SWINGLE (**6***a*), ALLEN (**1**), CARDIFF (**6**), and others among botanists, may be accepted, namely, that the maternal and paternal chromatin, which contribute to the fusion nucleus at fertilization, maintain their separate identity throughout the sporophyte generation, so that the act of fertilization is really only completed with the fusion of the

spirem threads in synapsis, as a preliminary to sporogenesis; then, as these authors point out, the synapsis stage becomes the critical point in the life cycle of the individual. It is conceivable that the maternal and paternal chromatin after entering the same nucleus in fertilization could continue in this condition throughout the sporophyte history without interfering with a development, but that the intimate union which occurs in synapsis, where an interchange of material between the parental idioplasms probably takes place, might lead to the development of "incompatibilities" between the plasms, such as are exhibited in irregularities and disturbances in the reduction divisions, and finally more or less complete failure of later development. It may be remembered in this connection that in *O. lata* no irregularities in the prophases were observed until after the contracted condition of the spirem.

This discussion is intended to serve merely as a partial analysis of the problem, of which it does not offer a solution. A larger body of facts is very desirable, correlating cytological studies of hybrids with observations of their variations and their relations to the parental forms in all their characters. It may then be possible to determine whether observable differences in the mechanism of inheritance as exhibited by the chromosomes occur in Mendelian, intermediate, variable, and other types of hybrids.

THE CHROMOSOMES AND MUTATION

It is believed that one of the most important discoveries connected with the investigation thus far has been the observation of certain chromatic bodies with a peculiar origin and history, which have been designated heterochromosomes. Since they have been found in the dominant *Lamarckiana* × *lata* hybrid, I believe it is probable that they will also appear in *O. Lamarckiana* itself. This point is to be investigated as soon as possible. If this surmise is found to be true, then the pollen mother cells in which these bodies occur must differ in chromatin content, and hence probably in hereditary value, from those in which they are absent, provided that pollen grains from both these classes of mother cells reach maturity. It is highly probable that the latter supposition is true in the *O. Lamarckiana* hybrid, for an abundant amount of pollen reaches maturity, so that in crossing

experiments fertilization is as easily accomplished as with pure *O. Lamarckiana* pollen; which increases the probability that no extensive deterioration of pollen grains occurs. POHL (**22**) states, however, that only about 70 per cent. of the pollen grains of *O. Lamarckiana* are perfect. Whether the deterioration is selective is of course not known.

The fact that the heterochromosomes originate from the spirem by the cutting off of a portion of its length after synapsis, and later degenerate, suggests that in this manner a portion of the chromatin is dispensed with in certain pollen mother cells, while in others where heterochromosomes do not occur it is all retained. Those who hold strictly to the individuality of the chromosomes, and that they represent in some way certain specific characters or groups of characters, would probably maintain that by this means the specific characters represented by the discarded chromatin are lost. LILLIE (**15**) expresses the view (which he strongly substantiates) that the chromosomes can represent only general characters belonging to the organism as a whole; such as color, stature, pubescence, etc. These would appear to correspond with the unit characters of DEVRIES, at least in many essentials, for instances of the latter as cited by DEVRIES in the Oenotheras are pigment (in *O. rubrinervis*), stature (*O. nanella* is a dwarf), and pubescence. This loss of chromatin in the germ cells might then appear to be a method of dispensing with certain unit characters of the organism. A great amount of additional work will be required, however, in the study of somatic characters in connection with chromosome morphology, before it will be possible to correlate these two categories of structure, if such a correlation exists.

ROSENBERG (**25**) has recently published a further paper on the Drosera hybrid, in which he finds that occasionally two pollen grains of a tetrad have the characters of one parent and two those of the other parent, the supposition being that this is preceded by segregation of the maternal and paternal chromosomes in the first reduction division. This may be considered a proof that the segregation of parental characters when it occurs takes place during the reduction divisions. In the light of such facts it seems highly probable that mutations in the Oenotheras will be found to originate during the

reduction mitoses, and perhaps from irregularities in the distribution of chromosomes. The results of the paper mentioned above would also appear to show that a relation exists between chromosome morphology and the morphology of the pollen grains.

The origin and early stages of some of the bodies classed as "idiochromosomes" by WILSON (**36**) are similar to those I have found in Oenothera. In *Lygaeus turcicus*, one of the Hemiptera, a large and a small idiochromosome are found in the prophase, the large one first appearing about the time of synapsis. Slightly later it is seen as a more deeply staining longitudinally split thread among the chromosomes, which are also thread-like bodies at this time. During this period a smaller, somewhat elongated idiochromosome is also present. The larger idiochromosome condenses to a globular shape and is for a time attached to the plasmosome, but retains a central cavity, which represents the original longitudinal split. This makes its appearance quite similar to those described in Oenothera. The later history of the idiochromosomes in Lygaeus and other Hemiptera however, is quite different from those of Oenothera, and need not be taken up here. Small chromosomes, which possibly correspond with WILSON'S *m*-chromosomes, are also occasionally found in Oenothera (*figs. 26, 27*).

Miss STEVENS (**28**) has described chromatic bodies in the insects which correspond with the heterochromosomes of Oenothera in parts of their history. In Stenopelmatus, the "sand-cricket," a chromatic "element *x*" appears in synapsis in the spermatocytes attached to an end of the spirem. It passes undivided into one of the spermatocytes of the second order and there degenerates during the resting stage between the maturation mitoses. Miss STEVENS believes this to be rejected chromatin, analogous to that observed in the ovogenesis of many forms. In the animal Sagitta, which is hermaphrodite, Miss STEVENS (**27**) found in spermatogenesis bodies which resemble the heterochromosomes of Oenothera in some respects. This "element *x*" appears in the spermatogonial divisions, where it divides before each mitosis. It also divides in the prophase of the first maturation division, and when the chromosomes are on the equatorial plate in metaphase these bodies are found in various

positions on the spindle. But they enter the daughter nuclei and divide again before the second spindle forms, and one of the products thus enters each spermatid. No such body is found in ovogenesis.

The only previous suggestion of heterochromosomes in plants is in a recent paper of CARDIFF (**6**), in which he mentions a heterotropic chromosome in the heterotypic mitoses of *Salomonia biflora*. He believes that this chromosome passes to one pole undivided in the first mitosis, but has not studied it further.

The difference found in the number of chromosomes in the mutants of Oenothera very strongly favors considering these forms of "specific" rank. I think it will be evident to any one studying carefully and comparing the different mutants that they are quite as distinct and easily distinguishable as are the species of any ordinary genus. The differences in the number of chromosomes is still further and, I think, conclusive evidence that the forms concerned are distinct "species."

It seems necessary to conclude, therefore, that the phenomena of mutation as described by DEVRIES in the genus Oenothera are either due to *O. Lamarckiana* being some peculiar type of hybrid in which the earlier crosses are appearing again in comparatively rare numbers; or on the other hand that some process of differentiation, the most probable seat of which is the germ plasm, has led to the production of distinct types of germ cells differing in chromosome morphology and in hereditary value. The middle ground, the assumption that these various mutants are merely the widely fluctuating variations of the original *O. Lamarckiana*, is believed to be no longer tenable.

Moreover, there are several lines of evidence, partly negative, which tend in various ways to discredit the possible hybrid origin of these forms. Some of these arguments will be mentioned.

1. It has been clearly shown by MACDOUGAL (**17**, **18**) and a number of collaborators that *O. Lamarckiana* was originally native to various parts of North America.

2. If the mutants always existed together with the parent form, it seems probable that some at least would have been recognized as species and preserved in European or American herbaria.

3. *O. biennis* and *O. grandiflora*, American members of the genus, have been shown by MACDOUGAL to be mutating.

4. Among all the known types of hybrids, with their widely varying methods of transmission and of variation, none are known, so far as the writer is aware, which present a series of phenomena of variation resembling or comparable to those found in *O. Lamarckiana* and its mutants.

5. Hybrids from crosses among the mutants of Oenothera follow the ordinary laws of hybridization; *e. g.*, *O. lata*×*O. Lamarckiana* is a Mendelian or dichotomous hybrid; and *O. nanella*×*O. rubrinervis* is a hybrid of intermediate type, which breeds true.

6. If *O. Lamarckiana* has itself been hybridized, where did the forms originate with which, on this assumption, it was hybridized? No scrap of evidence that they ever originated from any source except from *O. Lamarckiana* seed has been found. We must then assume that this is their only method of origin until the contrary is proved to be the case.

Summary[5]

1. Failure of pollen development in *O. lata* is not due to ingrowth of the tapetum to fill the loculus, as described by POHL, but to some other agency at work in the hybrid, the nature of which is not fully explained.

2. Pollen development in *O. lata* may proceed to the formation of tetrads, but degeneration of both mother cells and tapetum frequently begins in the resting stage or during the prophase of the first mitosis.

3. Heterochromosomes arise in *O. lata* in the prophase after synapsis. There may be one or two such bodies, which are formed as large rings by the cutting off of a portion of the spirem thread before the remainder breaks up into chromosomes. These bodies later are found in metaphase of the heterotypic mitosis, usually in the cytoplasm near the spindle. They do not divide, but become smaller and

[5] As this paper is passing through the press, conditions are being found in the pure *O. Lamarckiana* which will modify the interpretation of the bodies called heterochromosomes. The inference that *O. Lamarckiana* itself has the same number of chromosomes as the dominant *O. Lamarckiana* hybrid is also apparently not borne out by the facts. There seem to be variations in the number of chromosomes, which will require further careful study before an interpretation will be possible.

probably disappear at the end of the first mitosis. These bodies are also found in the *O. Lamarckiana* hybrid, in which they doubtless have the same origin. They probably represent discarded chromosomes, and this is perhaps a means of lessening the number of chromosomes in certain germ cells of the species. Some mother cells do not contain them. In such cells the (sporophyte) count of chromosomes in *O. lata* is fourteen and in the *O. Lamarckiana* hybrid probably twenty.

4. The occurrence of extra nuclei formed by chromosomes left behind in the cytoplasm in the tetrad divisions of the hybrid *O. lata*, as well as in a number of other hybrids recently described, puts under suspicion the purity of any other plant, such as *Hemerocallis fulva*, in which such extra nuclei are known to occur.

5. The inference seems justifiable that the mutations of *O. Lamarckiana* arise during the reduction divisions and that pollen grains which will give rise to mutants differ in their potentialities and probably also in chromatin morphology from the ordinary pollen grains of the plant.

These facts lead to the conclusion that a cytological basis may be found for the phenomena of mutation as observed in the Oenotheras.

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EXPLANATION OF PLATES II–IV

All the figures were drawn with the aid of a Bausch and Lomb camera lucida. A Zeiss 2^{mm} apochromat oil immersion lens was used, with Zeiss compensating oculars. The figures are all reduced one-half.

PLATE II

Oenothera lata (dominant)

FIG. 1. Transverse section of a loculus of an anther; pollen mother cells in "resting" stage. ×325.

FIG. 2. Slightly later stage; pollen mother cells passing from "resting" condition to early prophase; tapetal cells uninucleate. ×690.

FIG. 3. Later stage; pollen mother cells degenerating; tapetal cells binucleate, some of them degenerating. ×690.

FIG. 4. Pollen mother cell degenerating; the nucleus has disappeared and irregular deeply staining bodies appear in the cytoplasm. ×1500.

FIG. 5. A loculus after the pollen mother cells have disappeared; the remnants of the tapetum form a thin layer partly lining the cavity; the ingrowth of the middle layers has begun. ×325.

FIG. 6. A later stage; the middle layers have grown in, almost completely obliterating the loculus. ×325.

FIG. 7. Late stage in pollen development; the middle layers are being pressed together; the tapetal cells are narrow radially; the pollen grains are very irregular in shape. ×155.

FIG. 8. "Resting" nucleus of pollen mother cell; the nucleolus contains several highly refractive vacuoles; at the nodes of the reticulum are chromatic bodies, probably representing the "prochromosomes" of OVERTON. ×2125.

FIG. 9. Slightly later stage; the chromatic bodies are rounded in shape; safranin-gentian; 3.3 μ. ×2125.

FIG. 10. Slightly later stage; the reticulum is beginning to contract away from the nuclear membrane, which at this time is extremely delicate; 3.3 μ. ×2125.

FIG. 11. Spirem stage just previous to synapsis; four nucleoli are present; the nuclear membrane is extremely delicate; the spirem thread is very thin, about half its thickness subsequent to synapsis; Safranin-gentian; 4 μ. ×2125.

FIG. 12. Spirem thread contracted in synapsis; iron-haematoxylin-orange; 5μ. ×2125.

FIG. 13. Mother cell in synapsis; chromatic masses are scattered through the cytoplasm; iron-haematoxylin-orange; 7.5 μ. ×2125.

FIG. 14. Just after synapsis; loops of the spirem pass outwards to the wall, giving a false appearance of doubling; the nucleolus is flattened and appressed to the nuclear wall, which is more definite at this time; iron-haematoxylin-orange; 5 μ. ×2125.

PLATE III

FIGS. 15–24. *O. lata* (dominant).

FIG. 15. Nucleus at the time of synapsis; the nuclear membrane is very thick and irregularly indented; safranin-gentian; 4 μ. ×2125.

FIG. 16. Portion of spirem after synapsis; it is about double its former thickness and very much shorter; the loop (*a*) of the spirem may indicate the method of origin of a heterochromosome; safranin-gentian; 4 μ. ×2125.

FIG. 17. Slightly later stage showing a portion of the spirem after synapsis; the large ring body, which has been detached from the spirem, is a heterochromosome (*h*); the nucleolus is present and the nuclear membrane is becoming delicate previous to breaking down; safranin-gentian; 3.3 μ. ×2125.

FIG. 18. Section of nucleus showing two nucleoli and a heterochromosome (*h*); the adjacent section contains the spirem; safranin-gentian; 4 μ. ×2125.

FIG. 19. Similar section showing two heterochromosomes (*h*) and the nucleolus; safranin-gentian; 4 μ. ×2125.

FIG. 20. Late prophase, showing nucleolus, two heterochromosomes (rings), and seven other chromosomes with indications of pairing; safranin-gentian; 3.3 μ. ×1500.

FIG. 21. Later prophase showing thirteen chromosomes; the nucleolus and one chromosome on adjacent section; no heterochromosomes present; indications of pairing of the chromosomes; the cytoplasm is pushing into the nuclear cavity; iron-haematoxylin-orange; 7.5 μ. ×1500.

FIG. 22. Nucleolus and ten chromosomes; the stain is withdrawn from the shell of the nucleolus, except on one side; iron-haematoxylin-orange; 7.5 μ. ×1500.

FIG. 23. Metaphase of heterotypic mitosis, showing twelve chromosomes (some paired) and a heterochromosome (*h*); 5 μ. ×1500.

FIG. 24. Same stage as last, showing two heterochromosomes (*h*) in the cytoplasm, and ten chromosomes on the spindle; 5 μ. ×1500.

FIGS. 25–34. *O. Lamarckiana* (dominant).

FIG. 25. Section of heterotypic spindle, showing two heterochromosomes (*h*) of equal size on the same end of the spindle; 7.5 μ. ×1500.

FIG. 26. Same stage, showing chromosomes closely grouped in the equatorial plate; two heterochromosomes (*h*) of unequal size are present, and a very small chromosome (*m*) out of position on the spindle; iron-haematoxylin-orange; 7.5 μ. ×1500.

FIG. 27. Showing chromosomes closely grouped in the equatorial region of the spindle, and a small chromosome (*m*) nearer the pole; iron-haematoxylin-orange; 7.5 μ. ×1500.

FIG. 28. Showing two smaller paired chromosomes (*m*) at the side of the spindle; 7.5 μ. ×1500.

FIG. 29. Polar view of equatorial plate of heterotypic mitosis; one heterochromosome (*h*) present; 7.5 μ. ×1500. *Fig.* 32 is the adjacent section of this nucleus.

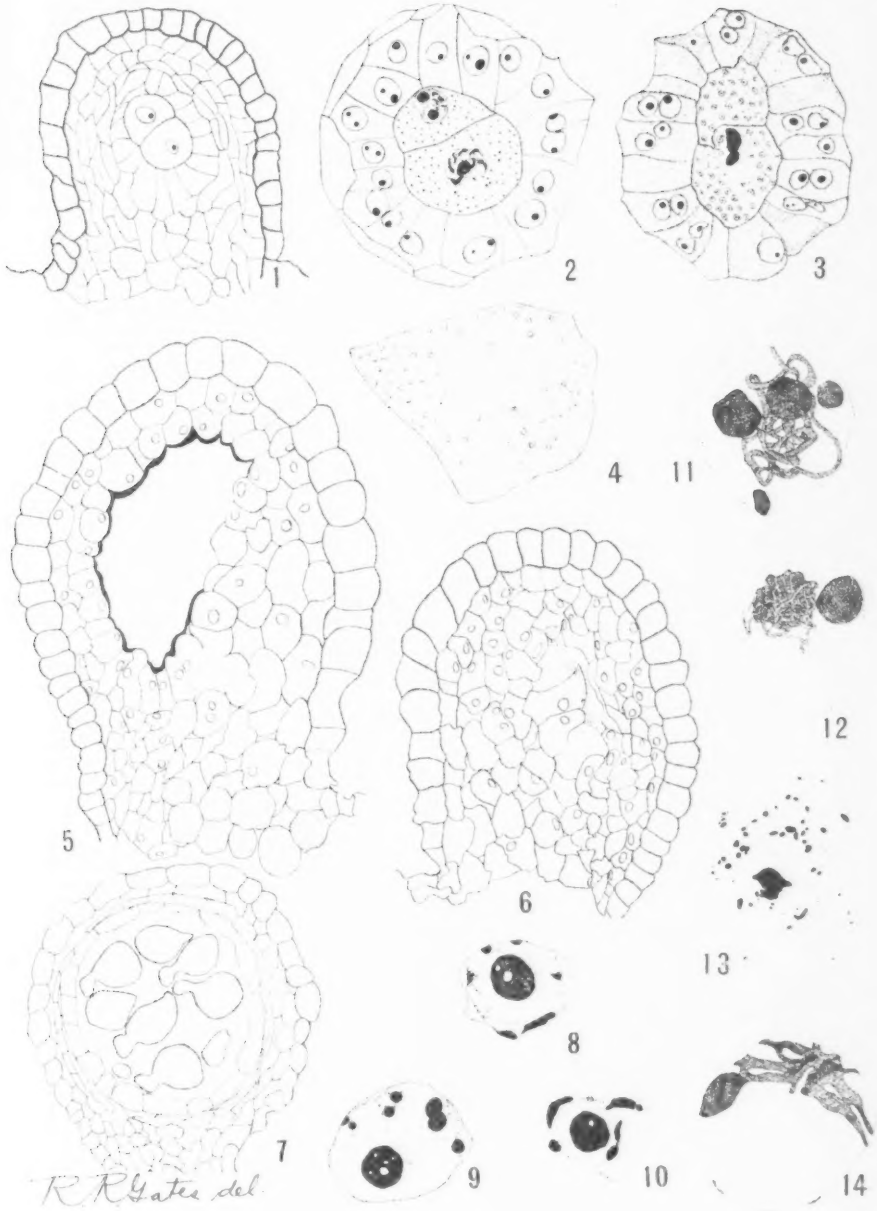
FIG. 30. Section of heterotypic spindle, showing a heterochromosome (*h*), an unequal pair of chromosomes, and an equal pair at the periphery of the cell. 7.5 μ. ×1500.

FIG. 31. Portion of the spindle, showing two chromosome dyads and a heterochromosome (*h*); 7.5 μ. ×1500.

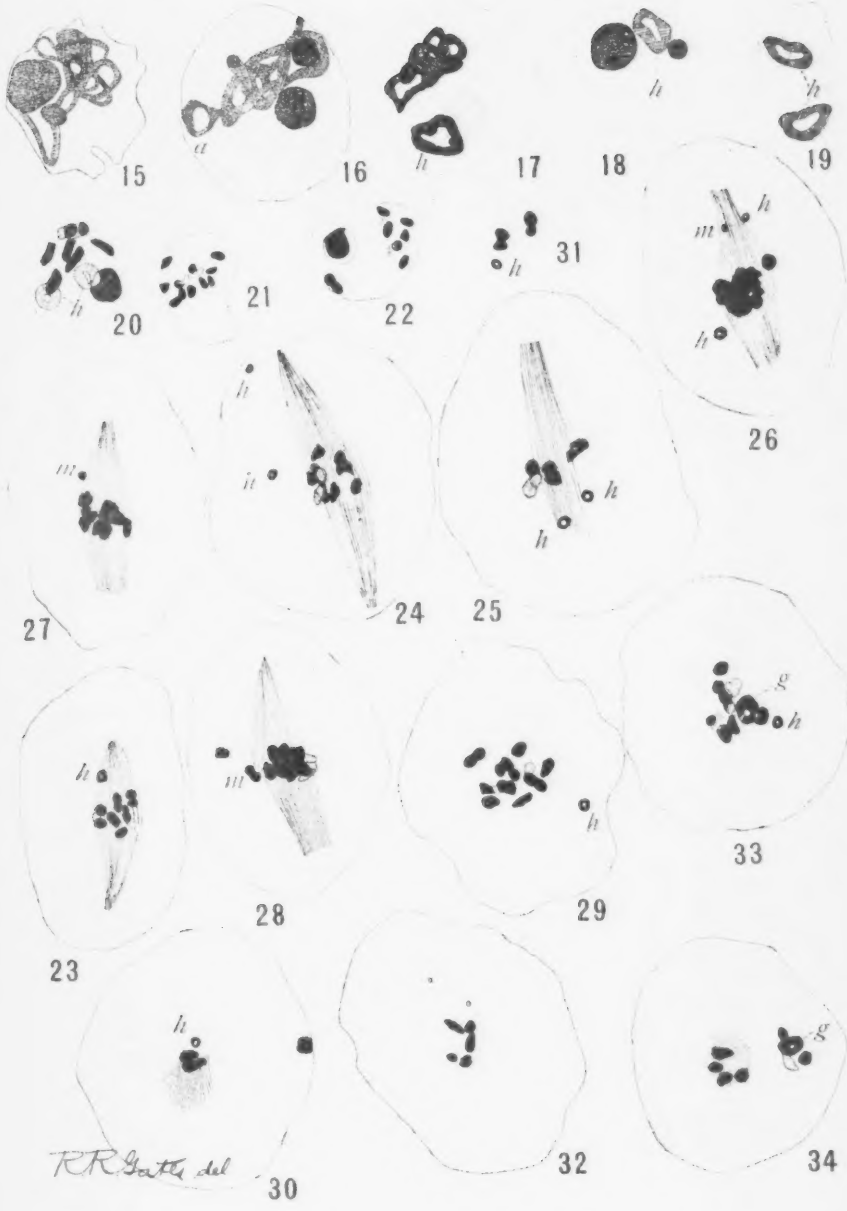
FIG. 32. Portion of the equatorial plate, showing two very small bodies which stain less deeply than the chromosomes and are probably remnants of a heterochromosome; iron-haematoxylin-orange; 7.5 μ. ×1500.

FIG. 33. Section of equatorial plate of heterotypic mitosis, showing a number of chromosomes, a heterochromosome (*h*), and a giant chromosome (*g*), which is very large and ring-shaped; 7.5 μ. ×1500.

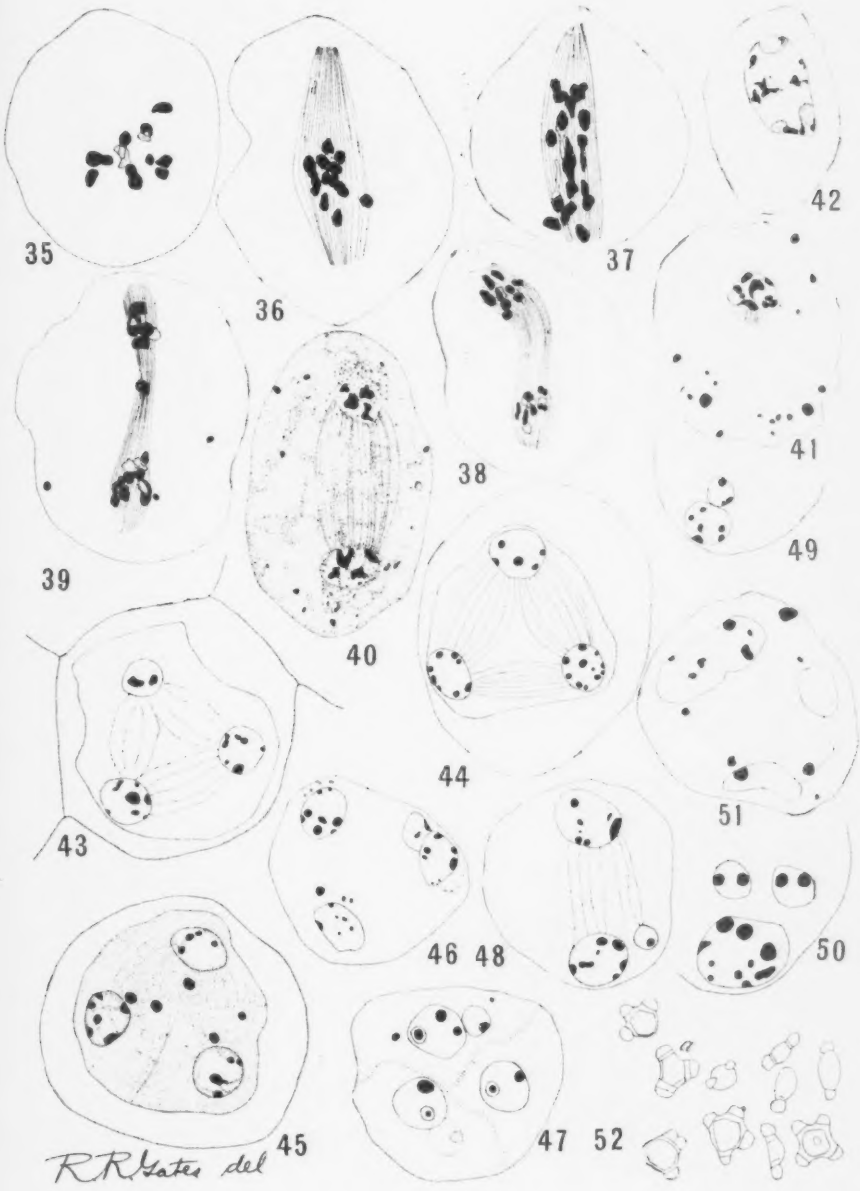
FIG. 34. Equatorial view of heterotypic spindle; the chromosomes at the side are displaced in the figure for the sake of clearness; one of them is the giant chromosome (*g*); 7.5 μ. ×1500.



GATES on OENOTHERA



GATES on OENOTHERA



GATES on OENOTHERA

PLATE IV

FIGS. 35–42. *O. Lamarckiana* (dominant).

FIG. 35. Another equatorial view; the unequal appearance of the members of a pair may be due to foreshortening. ×1500.

FIG. 36. Heterotypic spindle showing tendency of chromosomes to arrange themselves in groups of more than two. ×1500.

FIG. 37. Early anaphase, showing viscid consistency of some of the chromosomes; the number is at least twenty; no heterochromosomes present; iron-haematoxylin-orange; 7.5 μ. ×1500.

FIG. 38. Late anaphase, showing premature splitting of the chromosomes for the homotypic division; twenty chromosomes present; 7.5 μ. ×1500.

FIG. 39. Late anaphase; one chromosome lagging behind on the spindle, and several chromatic bodies in the cytoplasm; 7.5 μ. ×1500.

FIG. 40. Telophase in which the reconstruction of the daughter nuclei has begun; 7.5 μ. ×1500.

FIG. 41. One of the daughter nuclei in the early stages of reconstruction; remnants of the spindle still connect it with the sister nucleus; several large chromatic bodies are found in the cytoplasm; 7.5 μ. ×1500.

FIG. 42. Polar view of daughter nucleus in late stage of reconstruction; iron-haematoxylin-orange; 10 μ. ×1500.

FIGS. 43–51. *O. lata* (dominant).

FIG. 43. Pollen tetrad, showing three of the four nuclei; the latter vary in size and in chromatin content; the tetrads are still attached to each other at the ends, but free laterally; 5 μ. ×1225.

FIG. 44. Same as last, but nuclei of nearly equal size; safranin-gentian-orange; 5 μ. ×1275.

FIG. 45. Tetrad showing first appearance of walls; chromatic bodies in the cytoplasm; 5 μ. ×1225.

FIG. 46. Tetrad showing two extra nuclei attached to one of the daughter nuclei; 5 μ. ×1275.

FIG. 47. Tetrad after wall formation, showing extra nuclei, and chromosomes in the cytoplasm not surrounded by a nuclear membrane; 8 μ. ×1225.

FIG. 48. Section of a tetrad, showing a small extra nucleus, formed by one chromosome, connected by spindle fibers with one of the larger nuclei; 5 μ. ×1225.

FIG. 49. Portion of a tetrad showing a small extra nucleus beside one of the tetrad nuclei; 5 μ. ×1225.

FIG. 50. Portion of tetrad showing two small extra nuclei, each containing two chromosomes; 5 μ. ×2125.

FIG. 51. Tetrad showing irregular nuclei, and chromatin in the cytoplasm; 8 μ. ×1500.

FIG. 52. Young pollen grains of *O. Lamarckiana* (dominant); *a* shows a normal-shaped grain; grains showing various abnormalities, such as those illustrated, are of frequent occurrence. ×310.

DEVELOPMENT OF OVULE AND FEMALE GAMETOPHYTE IN *GINKGO BILOBA*

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY
XC

IDA ELEANOR CAROTHERS

(WITH PLATES V AND VI)

Ovules were collected at Elyria, Ohio, at intervals of two weeks, from the first of May until the latter part of August 1905, and were sent to Chicago packed in wet cotton. In April 1906, additional collections were made. Having been freshened by a stay in a moist chamber, the material was killed and fixed in chromacetic or chromacetosmic acid and put up in paraffin.

I wish to acknowledge my indebtedness to Miss A. M. STARR of Elyria, Ohio, for material, and to express my appreciation of the helpful suggestions of Professor JOHN M. COULTER and Dr. CHARLES J. CHAMBERLAIN of the University of Chicago, where the work was begun.

Megasporangium

DEVELOPMENT.—The megasporangia are borne on the short spur shoots, which bear also a few leaves, the longer shoots producing leaves only. By the first of April the terminal buds of the spur shoots have begun to swell slightly, and after removal of the brown bud-scales, the green leaves can be seen, but ovules are not yet distinguishable. Unopened buds collected the middle of April contain ovules which, with the stem bearing them, measure 2^{mm} in length. The ovules alone are only 0.25^{mm} long; they appear singly or in pairs at the end of leafless stems, and are pale cream color, while the leaves in the bud are bright green. Vertical sections (*fig. 1*) show the undifferentiated nucellus not yet wholly enclosed by the integument.

The buds soon open, and the leaves and ovule-bearing stems increase greatly in length. Not all the buds are fertile, but in those which are, the ovule-bearing stems vary in number from one to six. The ovules are still pale cream color, but show a faint green tinge which deepens rapidly as they enlarge. The ovule stalks appear, not at the growing tip of the spur shoot, but around it, as do the

strobili of most other gymnosperms. The succession of structures from without to the center of the bud is (*a*) brown bud-scales, (*b*) green scale-leaves, (*c*) foliage leaves, (*d*) ovules, (*e*) foliage leaves (*fig. 2*). Sections of these ovules show the nucellus enclosed by the integument but still entire, no tissue having broken down to form the pollen chamber.

At the beginning of May the ovules average 0.75^{mm} in length and are little if any greater in diameter than the stems that bear them. In some cases the nucellar beak protrudes as a tiny papilla from the micropyle. Vertical sections (*figs. 3, 4*) show the presence of a large pollen chamber, often containing pollen grains already developing tubes.

SPOROGENOUS TISSUE.—In ovules collected the latter part of April and the first of May, the sporogenous tissue has been differentiated, and all stages from immature mother cells to complete tetrads are to be seen. About at a level with the junction of the nucellus and the integument (*figs. 3, 4*) is an ovoidal mass of cells (*fig. 5*) which differ from those around them in being larger, having granular cytoplasm without vacuoles, and large nuclei with conspicuous nucleoli. All these have the appearance of sporogenous cells, although as a rule there is only one mother cell (*fig. 5*). In fifty ovules only one shows more than a single mother cell (*fig. 6*), that single exception possessing two.

The origin of the mother cell from a hypodermal cell could not be traced. Nor could it be determined whether the differentiation of the mother cell precedes that of the surrounding cells, in which case they would be purely tapetal in morphology as well as in function; or whether the whole mass is differentiated, the picking out of the functional mother cell occurring later. If the latter be the case, the mass is sporogenous, and the presence of such cells around the youngest mother cell observed strengthens the view that such is the case. The origin of the two mother cells and the cells between them from the same row (*fig. 6*) is another point in favor of this probability.

The mother cell is usually slightly below the center of the mass (*fig. 5*), and is distinguishable from the surrounding cells by its greater size, more deeply staining wall, and non-granular cytoplasm. At first it is non-vacuolate and the large nucleus is near the center

(*fig. 7*), but later a large vacuole appears below and sometimes a smaller one above the nucleus, the latter consequently taking a position nearer the top of the cell (*fig. 8*). The mature mother cell shows, in the cytoplasm below the vacuole, a peculiar kinoplasmic mass (*fig. 8*) similar to those already reported in the mother cells of Thuja (**7**), Taxodium (**1**), Larix (**6**), and Taxus (**2**), and in the eggs of Thuja and Taxodium. The significance of this mass has not been determined.

The nucleus soon goes into synapsis (*fig. 9*), the kinoplasmic mass disappearing about the same time. In the single preparation showing the next stage, the eight chromosomes, which result from synapsis, divide even before the disappearance of the nuclear membrane and entrance of the spindle fibers, although the latter are visible in the surrounding cytoplasm (*fig. 10*). The chromosomes give no indication of their second division, although in some forms such indications are usual. Repeated counting of chromosomes on spindles in various stages through the prothallium gave the constant number eight.

TETRAD.—The spindle of the first division is peculiar in being obliquely placed (*figs. 11, 12*), possibly as a result of the vacuole in the lower part of the cell, the kinoplasmic fibers extending only in the cytoplasm next the wall. The lower of the two cells resulting from this division is slightly larger (*fig. 6*) than its sister. It soon markedly exceeds the other cell, however, the dividing wall arching up into the latter (*fig. 13*). The division of the lower cell (*figs. 14, 15*) precedes that of the upper, which in some cases does not divide at all (*fig. 14*). Usually, however, a linear tetrad is formed (*fig. 16*), the lowest spore of which enlarges rapidly, absorbing the contents of the other three. Although the linear arrangement of the four spores is most common, other forms of tetrads were observed. One case of a bilateral tetrad (*fig. 18*), and two instances of the transverse division of the lower cell followed by the vertical division of the upper cell were found. In one of the latter the nucleus of the upper cell has divided, but the separating wall has failed to appear (*fig. 9*). In all cases the lowest spore, the one nearest the chalaza, enlarges before the upper ones do, and by absorbing material from them and monopolizing most of the material coming into the ovule prevents their further development. Similar to this earlier development of the lowest spore of the tetrad is

the development of the lower of the two mother cells in a single ovule (*fig. 6*). COULTER (**3**) suggests that this early development of the lowest cell may be the result of a favorable position in regard to nutriment coming up through the chalazal tissue.

The functional spore enlarges rapidly, its nucleus being held near the upper end of the cell by a large vacuole below. The presence of this vacuole in the spore suggests the probability of the parietal position of the free nuclei of the embryo sac from the very beginning. The appearance of a vacuole and the consequent parietal placement of the two nuclei resulting from the first division of the spore nucleus is reported by LAND in *Ephedra trifurca* (**8**); but in Ginkgo the vacuole is present even before the formation of the first gametophytic spindle, being in fact the same vacuole that was in the mother cell.

PROTHALLIUM.—*Free nuclear division.*—Ovules of two weeks later are somewhat larger, and the embryo sacs contain free nuclei (*fig. 20*), the number ranging from 16 to 64. At this stage the sac is very thin, staining no more deeply than does an ordinary cell wall, and the plasmic layer lining it is very delicate. The nuclei are well separated and each has a conspicuous nucleolus. They are lenticular in side view and circular in face view, and average 13.5 μ in long diameter by 8.14 μ in short diameter. In these early stages the division of the free nuclei is simultaneous, although each of several sacs with 64 spindles shows the latter in several different phases (*fig. 21*). This is of interest, inasmuch as later free nuclear division proceeds irregularly, nuclei in a single sac showing all conditions through the various stages of karyokinesis and resting (*fig. 24*.) At first the nuclei of such sacs are definitely placed, so that although all stages are to be seen in the sac, the nuclei in any given portion are in the same stage. Later the sacs show nuclei in all stages grouped without regard to condition.

Free nuclear division continues from the second week in May until the first week in July, the whole ovule meanwhile enlarging and the embryo sac growing rapidly in both size and thickness. The protoplasm becomes granular and the free nuclei divide so rapidly that they decrease in size (*figs. 22, 24*). By the first of July the cytoplasm has become very granular (*fig. 23*), and the number of nuclei

large, the numerous divisions and their irregularity making the number larger than 256, which is so common a number among the gymnosperms. It is not impossible that the irregularity in division of the free nuclei, varying as it does from the simultaneous division said to obtain in other forms, may have been due to the unnatural conditions —growth after separation from the tree—although an effort was made to render conditions as natural as possible.

Wall formation.—About this time there appears on the outer surface of the plasmic sac a delicate membrane (*fig. 24*). This is not a *Hautschicht*, nor is it in any way attached to the original spore wall or embryo sac. It is a true cell wall of the type known as walls of deposit, and is formed by the protoplasm which lines it. Then walls appear in the cytoplasm, perpendicular to this enclosing membrane and with their outer edges fastened to it (*fig. 25*). The current accounts of wall formation following free nuclear division in the embryo sac, beginning with Miss SOKOLOWA'S (**10**) description and continuing through subsequent papers by other investigators, state that the walls appear at right angles to the embryo sac or spore wall, and with their outer edges fastened to it. This is distinctly not the case in Ginkgo; and the ease with which the spore membrane may be peeled away from the prothallia of many other gymnosperms, even when the latter are but partly developed, suggests the probability that it is not the case in them.

It has long been known that the walls of microspores are quite separate and distinct from the wall of the mother cell in which they lie, and that the spores when mature escape from the mother cell membrane through ruptures or by its solution. In sectioned microsporangia showing developing tetrads, these separate surrounding walls of the mother cells may be readily seen. It is known also that the outer portion of the microspore wall, that next the wall of the mother cell, is formed by deposit, and not upon spindle fibers, as are the walls between the spores themselves. The megaspore wall, as can be seen in the figures showing the tetrad formation, with the exception of that comparatively small portion which divides it from the non-functional spores, is the old membrane of the mother cell and corresponds to the mother cell wall enclosing the microspore tetrad. The formation of this separate membrane upon the outer surface of

the sac by deposit, therefore, corresponds to the formation of the walls of the microspore, and the fastening to it of the outer ends of the radial walls corresponds to the similar attachment of the walls cutting off the prothalial cells in the male gametophyte.[1]

These walls, at right angles to the enclosing membrane and fastened to it, separate the nuclei, probably each one being enclosed in a cell of its own. In other forms the first radial walls are said to come in on the spindles of the last simultaneous free nuclear division. Since the later divisions of the free nuclei here are far from simultaneous, this cannot be the case. Although the coming in of these first walls was not observed, the subsequent centripetal growth and division of the cells formed by their development are clearly seen (*fig. 25*). The inner cells of the centripetal rows are larger than the outer ones, the size of the latter being early diminished by repeated divisions, both tangential and radial (*fig. 25*). In most cases the nuclei of these cells, which are open to the interior of the sac (*fig. 27*), are much larger than those of the enclosed cells near the periphery. Most of the open cells have a single nucleus (*fig. 27*), but two nuclei are to be seen in some (*fig. 28*), and in a few, three nuclei. Binucleate cells (*figs. 29, 30*) are common in the tissue near the open central space, and even a few multinucleate cells are present (*fig. 31*), but since cells with a single nucleus are not found in the mature endosperm, these very cells must later become uninucleate. Whether this change results from a fusion of the several nuclei, a degeneration and absorption of the superfluous number, or their separation by the formation of intervening walls is not certainly known. In all cases of wall formation seen within cells, however, the wall is on a regular karyokinetic spindle. Usually the two nuclei in a cell are in contact, and it is not uncommon for one of them to be smaller and more dense than the other, suggesting its degeneration and absorption (*fig. 30*). From this it appears probable that cells which have at first two or more nuclei become uninucleate by a fusion of the several into one, or by the degeneration and absorption of the superfluous ones, and not by the formation of separating walls.

[1] One case of curious and irregular wall formation, abnormal and perhaps the result of pathological conditions, is seen in a sac of June 19. The plasmic layer containing free nuclei is as usual, but in addition there are eight little groups of nuclei, each group quite separate from the others and each with an inclosing membrane (*fig. 26*).

Wall formation in the cells open and growing toward the center, which is filled with sap, is carried on in the usual way at the peripheral end of each new cell, by the formation of a nuclear plate on the spindle of the division by which the new nucleus is formed (*fig. 32*).

The mode of formation of the side walls of these same cells, however, is not so easily determined. Curious double spindles (*fig. 33*) and extra fibers radiating from nuclei in the last stages of karyokinesis (*fig. 32*) indicate some such procedure as this: When a nucleus divides, lengthening the centripetal row, fibers radiate from it, not only to the sister nucleus, but radially as well, connecting with similar fibers radiating from other nuclei, and upon the resulting spindles are formed the plates which later develop into walls. This mode of wall formation has long been known in the endosperm of many angiosperms. In some cases are to be seen similar double spindles which lack nuclei at the two ends, the single nucleus concerned being in the middle. Further work is necessary to settle this point.

Centripetal growth continues through July and August, being most active at the base and filling the central cavity with tissue by the last week in August. In shape the prothallium is almost ellipsoidal, being slightly flattened on two opposite sides (*figs. 34, 35*). As a result of the shape and the equal growth from the sides, the closure of the tissue is in a plane parallel with the broader sides of the gametophyte. Only in sections cut at right angles to the flattened sides does the closure appear as a line (*fig. 34*). Upon approaching each other the open, centripetally growing cells of opposite sides do not unite and form a common end wall, as they are said to do in many other forms. Instead, each cell forms an independent end wall, separate from that of the other cells (*fig. 36*). The resulting body is not a solid mass, but a tissue which may be easily opened at the middle. Later these separate end walls, lying as they do against each other, may so unite as to appear and really form a common wall, but such is not the case in the oldest prothallia examined.

Growth.—Simultaneous with centripetal growth, the whole prothallium increases in bulk by growth and division of cells, the inner ones enlarging greatly and those at the periphery continuing meristematic (*figs. 25, 38*).

Archegonium initials appear very early; the two-celled neck has

been formed and the central cell has enlarged considerably before the tissue lining the sac equals in depth the width of the central cavity (*fig. 37*). HIRASÉ (**4**) has described the development of the archegonia.

The outer wall of the prothallium, the first wall built by the plasmic sac, becomes much thickened, and the megaspore membrane or embryo sac becomes exceedingly thick and dense, making the entrance of the killing and the fixing fluids difficult. The structure and development of the megaspore wall is set forth in a paper by THOMSON (**11**), but as my results differ slightly from his, I shall give them. THOMSON states the thickness of the megaspore coat from a mature seed to be 4.5–5 μ. The wall shown in *fig. 22* measures 2.1 μ in thickness and that of an endosperm which has just closed in the center measures 6.16 μ. At first the membrane, which as shown above is in reality the mother cell wall, is thin and delicately granular (*fig. 20*). It rapidly thickens and becomes coarsely granular. A cross-section shows a very thin inner layer acting as a base for the transversely placed rods of the thick outer layer (*fig. 22*). THOMSON reports these rods as being quite irregularly placed. Subsequent thickening takes place in the outer layer only, the rods increasing in length and thickness, and the thin inner layer finally disappearing (*fig. 25*). Not infrequently, in material of this stage, little bunches of the rods forming the wall are found torn out and scattered over the slide. The result of the ease with which the outer layer is torn across between the rods and the resistance offered by the thin inner layer is shown at the upper end of the wall in *fig. 22*. The ends of the rods, presented at right angles at the surface of the spore coat, are the cause of the slight roughness there. After the disappearance of the smooth inner layer, the inner surface becomes similarly rough.

The complete independence of the heavy embryo sac and the outer wall of the prothallium explains the position of the former with reference to the archegonial chamber of the mature endosperm. Being entirely free from the tissue of the endosperm, it is lifted up by the growth of the tissue around the archegonial chamber until it is some distance from the floor of the latter and forms a roof over it.

From early in May the ovules are green throughout, but as they increase in size the inner tissue becomes hyaline, the chlorophyll being

abundant in one place only—a shallow region at the surface of the single heavy integument. Soon after the appearance of walls and long before the filling of the sac with tissue, the gametophyte becomes green. In a few weeks it is by far the greenest thing in the ovule. An alcholic solution of this green pigment gives the spectrum of chlorophyll. The presence of chlorophyll, evidently functional, within a gametophyte wholly enclosed within an ovule has not been reported before. The thin walls of the cells of the integument, the paucity of chlorophyll there, and the presence of numerous large cavities full of a clear viscid liquid favor the transmission of light to the gametophyte, and, as a meristematic tissue, it responds by the formation of the pigment. WARMING (**12**) reports that the endosperm of *Cycas circinalis*, if fertilization fails to take place, sometimes grows out through the micropyle and in the light becomes green. No mention, however, is made of its becoming green before protruding.

The cells of the endosperm early fill with starch, the large grains characteristic of the storage forms being abundant in the inner cells (*figs. 26–38*), while smaller grains in all stages of formation are to be seen in the periphery.

Some of this starch is undoubtedly manufactured by photosynthesis within the prothallium, but some may be the result of absorption from surrounding nucellar and integumentary tissues. Starch is plentiful, however, only in the outer layers of integument, never close to the endosperm.

Spongy tissue

The spongy tissue, in the midst of which the mother cell appears, performs an important function in the nutrition of the prothallium. As the megaspore mother cell forms the tetrad, the whole spongy mass increases in bulk, the individual cells multiplying by spindles at right angles to the periphery of the mass (*fig. 39*). The nucellar tissue immediately surrounding at once shows signs of being absorbed, the protoplasmic contents of the cells becoming dark and granular even before the walls suffer collapse (*fig. 39*). This mode of increase in bulk of the spongy tissue and the adaptation of the surrounding nucellar tissue is described at length by Miss FERGUSON (**4**) in her work on Pinus. The result of this activity is a growing mass, at first

ovoidal, then ellipsoidal, of glandular cells encroaching upon and absorbing the surrounding nucellar tissue, the whole being enclosed in an enlarging cavity whose wall is made of elongating dividing cells. Although at first densely granular and non-vacuolate (*figs. 5, 6*), the absorbing cells soon become vacuolate (*fig. 18*), then multinucleate (*fig. 22*). Whether these nuclei are the result of direct or indirect division is not known. That the former is probably the case is suggested by the occurrence of such division in the absorbing cells of other sporangia. Moreover, spindles are seen in early stages only, and in those cases a plate is always present.

Until the fourth week in June the spongy tissue cells show great activity (*fig. 22*) in encroaching upon and absorbing the adjacent tissues, but soon afterward they themselves show signs of being absorbed by the enlarging prothallium (*fig. 24*), the latter being still in the free nuclear stage, but having formed the enclosing wall.

By the time the radial walls are formed and centripetal growth has brought the tissues one-third of the distance across the sac, the spongy tissue cells have been absorbed (*fig. 25*), their remains being only a thin mass of collapsed and heavily staining walls. The embryo sac now lies against the undifferentiated nucellar tissue, separated from the living cells of the latter only by this mass of dead cells which have given up their substance. When the prothallium has become a mass of tissue, it is quite near the surface of the nucellus, instead of far below it as at first. Most of the upper part of the nucellus has been absorbed, and the nucellar beak, at first so conspicuous, has collapsed (*fig. 34*).

Integument

The tissue of the single, thick integument is homogeneous at first, but by the last of May it is differentiated into three distinct tissues (*fig. 40*): an outer layer of large thin-walled cells with many mucilage-filled cavities; a middle layer of small, isodiametric cells; and an inner region of large, very thin-walled cells loosely held together. This inner layer is further differentiated into an outer layer having cells transversely elongated, and an inner layer having cells vertically elongated. This innermost layer of vertically elongated, delicate-walled cells appears only in the free portion of the integument, but the

remaining layers extend through its entire length, being the same both where it is separate from the nucellus and where it is continuous with it. The outer of these three layers becomes the fleshy, juicy part of the ripened fruit (*figs. 34, 35*); the middle layer, by a great thickening of walls and compacting of cells, becomes the stony coat; the inner fleshy layer, which is delicate and watery in early stages, becomes crushed and dry, forming the papery layer which lines the stony coat and adheres to it in the mature fruit. The nucellar tissue is quite distinct from the watery tissue of the integument, and in the upper part the two are not even in contact (*fig. 40*), so the former cannot form the papery coat as it is said to do by SEWARD and GOWAN (**9**). Below the line of junction of the nucellus and integument the former may contribute some small part to the papery coat, but it is almost entirely absorbed by the prothallium.

At the base of the ovule two bundles enter the watery tissue, coming up through a little gap in the tissue of a stony coat (*fig. 40*). Their course lies along the inner surface of the watery tissue, one curving up on each side and ending just below the point at which the integument becomes free from the nucellus. The abundant liquid in this watery tissue doubtless comes up through these bundles. Upon removal of the integument, which splits readily into two lips, the nucellus is seen to be flattened on two opposite sides, the intervening sides being angled (*figs. 34, 35*). It is in these angled sides that the bundles end (*fig. 35*). In some cases there is a three-lipped integument and a three-angled nucellus, there being also three bundles, one ending in each angled side.

SUMMARY

1. The ovule has a conspicuous nucellar beak and pollen chamber.
2. The sporogenous tissue is deep within the nucellus.
3. Differentiation of the mother cell has taken place by the first of May.
4. One mother cell is usual, but more may occur.
5. A peculiar kinoplasmic mass is present in the mother cell.
6. The gametophyte number of chromosomes is eight.
7. The first spindle is obliquely placed in the mother cell.
8. The tetrad may be complete or incomplete.

9. The tetrad is usually linear, but sometimes bilateral, or a combination of bilateral and linear.

10. The lowest spore is the functional cell.

11. The spore is vacuolate from the first and the free nuclei of following stages are probably always parietally placed.

12. Free nuclear division is at first simultaneous but gradually becomes irregular.

13. Free nuclear division extends, approximately, from the second week in May until the first week in July.

14. The cytoplasm, at first delicate, becomes granular and forms upon its outer surface a delicate wall.

15. Centripetally growing walls are formed between the nuclei and with their outer edges fastened to the membrane developed at the outer surface of the plasmic sac.

16. The sac fills with tissue by centripetal growth and division of cells, the inner ends being open.

17. These open cells are usually uninucleate but sometimes multinucleate.

18. Binucleate and multinucleate cells are frequent in young prothallial tissue, but later they become uninucleate, probably by fusion of the several nuclei or by degeneration of the superfluous ones.

19. When the centripetally growing cells meet at the center each forms an independent end wall.

20. The megaspore wall and the outer wall of the prothallium become much thickened.

21. The megaspore wall is composed of an inner, thin, firm layer, and a very thick, outer layer made up of rods formed at right angles to the surface.

22. The archegonia are quite far developed while there is still a large central cavity.

23. The gametophyte develops abundant chlorophyll, becoming the greenest tissue of the ovule.

24. The spongy tissue surrounding the mother cell is tapetal in function, absorbing the surrounding tissue, and finally being itself absorbed by the growing prothallium.

25. The spongy tissue cells become vacuolate and multinucleate.

26. The integument is early differentiated into three distinct

tissues, which become respectively the fleshy coat, the stony coat, and the papery coat.

27. Abundant liquid is brought to the watery layer by two bundles which enter through a gap in the stony coat and terminate just below the point of separation of the integument and nucellus.

CHARLESTON, ILL.

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EXPLANATION OF PLATES V AND VI

With the exception of *figs. 3, 4, 34, 35, 37,* and *40,* the figures were drawn with the aid of an Abbé camera lucida and all were reduced one-half in reproduction.

PLATE V

FIG. 1. Vertical section of a single ovule; April 15, 1906. ×15.

FIG. 2. Habit sketch of spur shoot, showing opened fertile bud; April 25, 1906. ×2.

FIG. 3. Vertical section of a pair of ovules; May 1, 1905. ×12.

FIG. 4. Vertical section of a single ovule; May 1, 1905. ×12.

FIG. 5. Vertical section through sporogenous mass with single mother cell; May 1, 1905. ×325.

FIG. 6. Vertical section through sporogenous mass with two mother cells; May 1, 1905. ×325.

FIG. 7. Young mother cell; May 1, 1905. ×650.

FIG. 8. Mature mother cell, showing vacuoles and kinoplasmic mass; May 1, 1905. ×650.

FIG. 9. Mother cell in synapsis; May 1, 1905. ×650.

FIG. 10. Mother cell just after synapsis, showing the eight chromosomes already divided for the heterotypic division, and the spindle fibers in the cytoplasm; May 1, 1905. ×650.

FIG. 11. Mother cell showing oblique spindle of heterotypic division; May 1, 1905. ×650.

FIG. 12. Mother cell showing oblique division; May 1, 1905. ×650.

FIG. 13. First division of mother cell completed; May 1, 1905. ×650.

FIG. 14. Lower cell showing spindle of homotypic division; upper cell preparing for division; May 1, 1905. ×650.

FIG. 15. Division of lower cell completed; May 1, 1905. ×650.

FIG. 16. Incomplete tetrad; upper cell failing to divide; May 1, 1905. ×650.

FIG. 17. Linear tetrad; lowest cell enlarging; May 1, 1905. ×650.

FIG. 18. Bilateral tetrad; May 1, 1905. ×650.

FIG. 19. Combination linear and bilateral tetrad; wall failing to appear in upper cell after vertical division of nucleus; May 1, 1905. ×650.

FIG. 20. Vertical section of a sixteen-nucleate sac and tapetum; May 18, 1905. ×225.

PLATE VI

FIG. 21. Portion of sac showing spindles of free nuclear division in slightly different phases; May 18, 1905. ×650.

FIG. 22. Portion of embryo sac showing granular cytoplasm, free nuclei, and multinucleate tapetal cells encroaching upon nucellus; June 5, 1905. ×650.

FIG. 23. Face view of plasmic sac with free nuclei; June 5, 1905. ×650.

FIG. 24. Portion of embryo sac showing absorption of tapetum; free nuclei in different conditions; and a cell wall developed upon the outer surface of the plasmic sac; June 20, 1905. ×650.

FIG. 25. Section of prothallium, showing centripetal walls fastened to outer membrane which is separate and distinct from embryo sac; July 19, 1905. ×325.

FIG. 26. Peculiar case of wall formation; June 5, 1905. ×650.

FIG. 27. Open cell from inner end of centripetally growing row; July 19, 1905. ×325.

FIG. 28. Open inner cell with two nuclei; July 28, 1905. ×325.

FIG. 29. Binucleate cell from prothallial tissue; July 28, 1905. ×325.

FIG. 30. Binucleate cell from gametophyte; one nucleus apparently undergoing absorption; July 19, 1905. ×325.

FIG. 31. Multinucleate cell from prothallial tissue; July 28, 1905. ×325.

FIG. 32. Open inner cell showing centripetal growth by means of spindles; extra fibers being attached to outer nucleus; July 19, 1905 ×325.

FIG. 33. Curious incomplete double spindle from open inner cell; July 19, 1905. ×325.

FIG. 34. Vertical section of ovule, showing embryo sac filled with tissue; the closure appearing as a line; August 21, 1905. ×1.

FIG. 35. Transverse section of ovule, showing embryo sac filled with tissue; showing radiation of cell rows from line of closure; August 21, 1905. ×1.

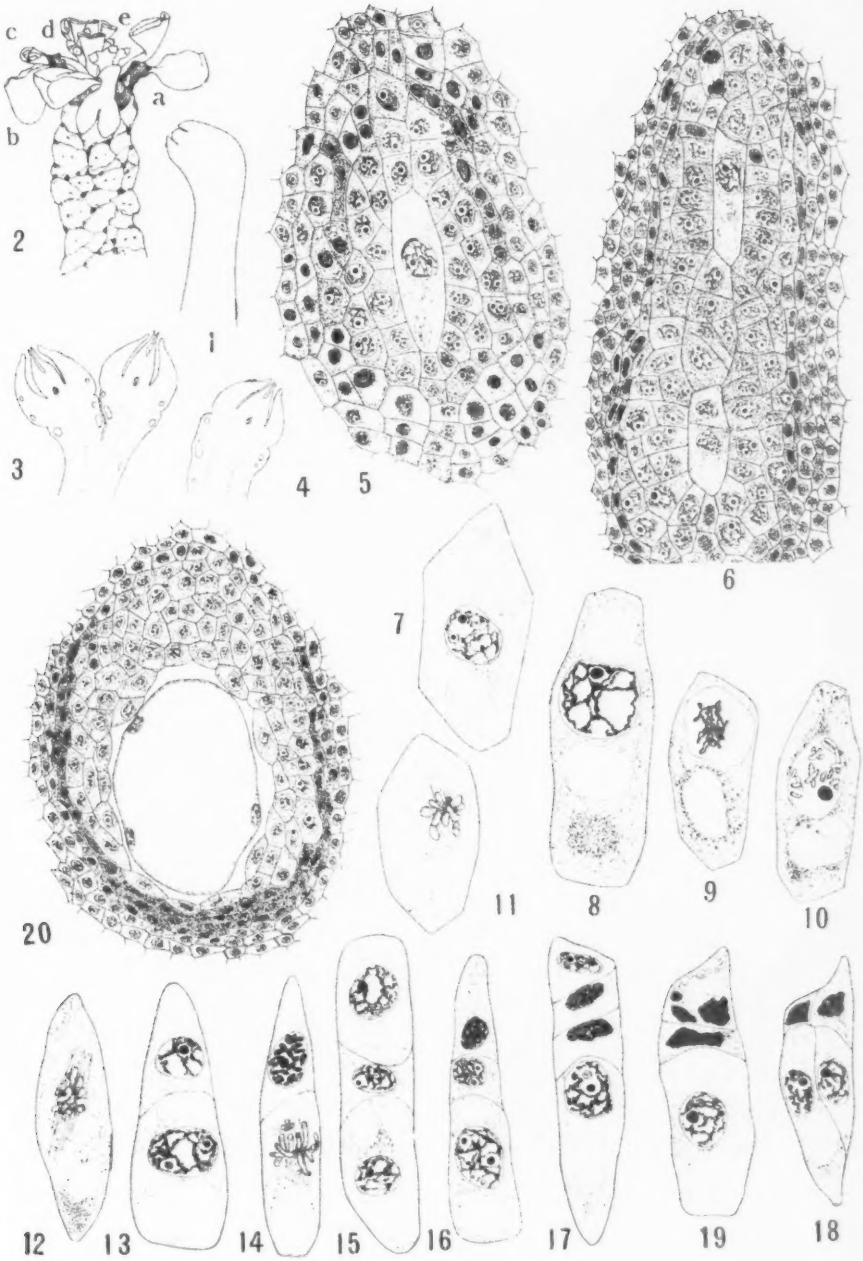
FIG. 36. Vertical section of tissue at line of closure, showing independent end walls of opposite cells; August 21, 1905. ×325.

FIG. 37. Diagram of vertical section of prothallium, showing amount of tissue present at time when archegonia are becoming conspicuous; July 19, 1905. ×2.

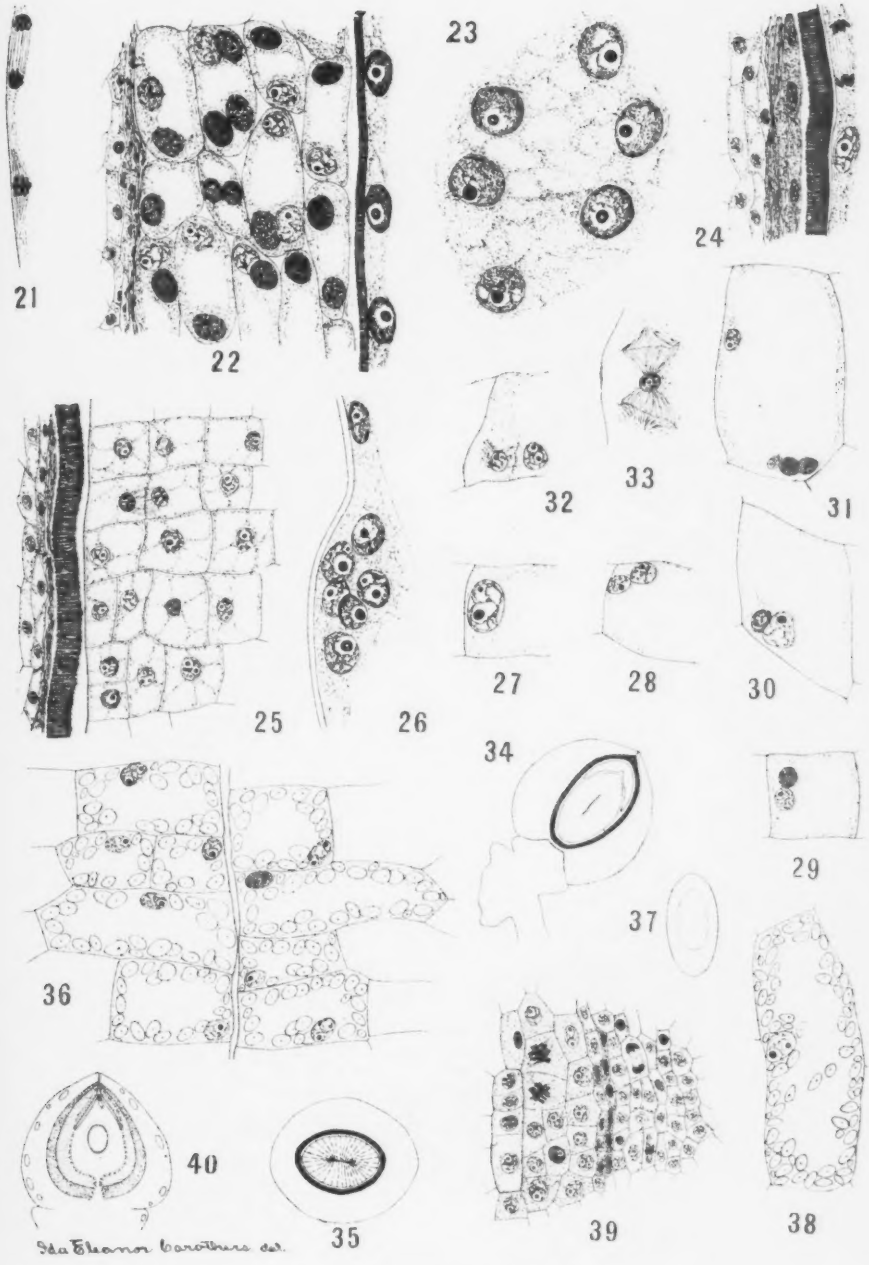
FIG. 38. Typical mature cell from gametophyte, showing single nucleus and abundance of starch; August 21, 1905. ×325.

FIG. 39. Vertical section through spongy mass, showing mode of increase in size, and effect upon surrounding cells; May 1, 1905 ×325.

FIG. 40. Diagram of vertical section of ovule, showing differentiation of tissues in the integument; June 10, 1905. ×4.



CAROTHERS on GINKGO



CAROTHERS on GINKGO

MORPHOLOGY OF SPATHYEMA FOETIDA

JAMES ELLIS GOW

(WITH SEVEN FIGURES)

During the late winter and the spring of 1906 the writer collected, in the vicinity of Blairstown, N. J., the material on which the following observations are based. The anthers of Spathyema and the portion of the receptacle in which the ovary lay imbedded were cut out, killed, and fixed in picracetic,[1] chromacetic, or picric acid, imbedded, sectioned, and mounted in the usual manner. The stains used were safranin, Delafield's haematoxylin, and Haidenhain's iron-alum haematoxylin. Of these the last gave by far the best results.

During the summer the buds which give rise to the plants of the next year develop on the thick underground rootstocks. By November the shoots are several inches in length. The flower buds occur as lateral offshoots of the leaf bud, in the outer integuments of which they are enclosed. Only one flower makes its appearance in connection with each shoot. Occasionally this one is lacking, and the leafy shoot appears alone.

THE MICROSPORANGIUM.

During February growth is resumed in the flower bud. At this time the single anatropous ovule makes its first appearance in the form of a group of cells projecting obliquely downward into the cavity of the ovary. The stamens appear as short projections from the surface of the receptacle. The filaments do not begin to lengthen until considerably later. The anthers at this stage of development have not attained their full size, but the four microsporangia and the connective may be readily distinguished by the naked eye. Upon making a microscopic examination of the anther in cross section it is seen that the various portions are already well differentiated. Through the middle of the rather thin-walled cells forming the connective

[1] A saturated solution of picric acid to which is added 1 per cent. of glacial acetic acid.

runs a well defined vascular bundle. The whole anther is invested by a layer of epidermal cells whose outer surface is somewhat thickened. In the interior of each microsporangium is a mass of thin-walled sporogenous cells (usually fifteen to twenty), indicating that the archesporial cells have developed some time before. These are surrounded by a well-defined tapetal layer. The four microsporangia are borne on the ventral surface of the stamen.

During the latter part of February and the first week in March the anthers increase greatly in diameter. This increase does not take place in the length of the connective so much as in the size of the four microsporangia. The connective appears to have reached its full development earlier. The growth of the microsporangia is accompanied by rapid increase in the number of sporogenous cells. While in a resting condition, the sporogenous cells differ from the surrounding cells in their greater size, in their generally hexagonal shape, and in the fact that the nucleus is larger in proportion to the size of the cell and shows a more distinct chromatin network than does the nucleus of the vegetative cell. Division of the sporogenous cells continues until they form, taken together, two-thirds of the diameter of the microsporangium. The last cells formed are considerably larger than those formed in the earlier stages of the process.

While this is going on, the filaments begin to lengthen out and push the anthers up until they press against the infolded edges of the perianth. By the second or third week in March the divisions of the sporogenous cells are completed. The mature pollen mother cells thus formed are from one and a half to two times the size of the cells from which they have descended. The tetrad formation is as usual. The walls of the spores are greatly thickened and do not stain readily with any of the anilin stains.[2] The cytoplasm is granular and the nucleus ill-defined. From the difficulty experienced in finding material in this stage of development, it is evident that it is very evanescent.

An examination of the pollen grains in water shows that the exine

[2] Staining the tetrads with haematoxylin was not attempted. Sections fixed in picric acid and stained with eosin showed the fibrous portion of the anther stained bright orange, mature pollen grains deep red, tetrads light yellow.

is of appreciable thickness (though much thinner than the wall of the cell in which it was developed) and is covered with minute spiny projections. The vegetative and generative nuclei appear faintly when the interior of the grain is brought into focus. When the pollen grain is treated with a solution of iodine green they appear much more clearly, and it is seen that the latter is much the larger of the two. The division between the two takes place just before the expulsion of the pollen grain from its investing cell.

After the expulsion of the pollen grains the collapsed mother cells may be seen lying about and partially filling the cavity of the anther; but in a day or two they break down and disappear. Simultaneously with the disappearance of the mother-cell walls, the tapetal layer breaks down and the contents of the cells become scattered throughout the cavity of the microsporangium. By the beginning of the last week in March the microsporangium is filled with a mass of mature pollen grains. In the meantime the filaments, as a rule, have lengthened sufficiently to push the anthers out past the infolded edges of the perianth. When this has taken place the anthers dehisce and the pollen grains are set free. Frequently, however, the lengthening of the filament is delayed and the mature pollen grains remain in the anthers for a week or more before they are finally set free. This is a phase of the subject that must be reserved for future study, but it would seem that the development of the filament and dehiscence of the anther are dependent to a certain extent on weather conditions. Probably, too, there is a correlation between their development and the development of the pistil. The dehiscence is along two longitudinal creases, each one of which separates the two microsporangia lying at the same end of the connective.

THE MEGASPORANGIUM

Material gathered early in February shows the ovary as a tiny cavity in the tissue of the receptacle, beneath the short thick style. The ovule at this period appears as a group of thin-walled, undifferentiated, strongly nucleate cells, projecting downward into the ovary and nearly filling it. As yet there is no distinction of nucellus and integuments. The style has pushed up far enough to bring the partially developed stigmatic cells even with the edges of the sepals.

By the last week in February the cavity of the ovary has considerably increased and made room for the growing ovule. At this point the differentiation between the nucellus and its integuments begins to be noticeable, but as yet there is no division between inner and outer integuments. By the first or second week in March the outer integument begins to appear, and the inner integument grows up so as nearly to cover the nucellus, leaving only a narrow micropyle above its tip. An examination of the nucellus at this stage shows that it is invested by a distinct outer layer of cells differing from the interior cells in their greater regularity of outline. The cell at the tip of the

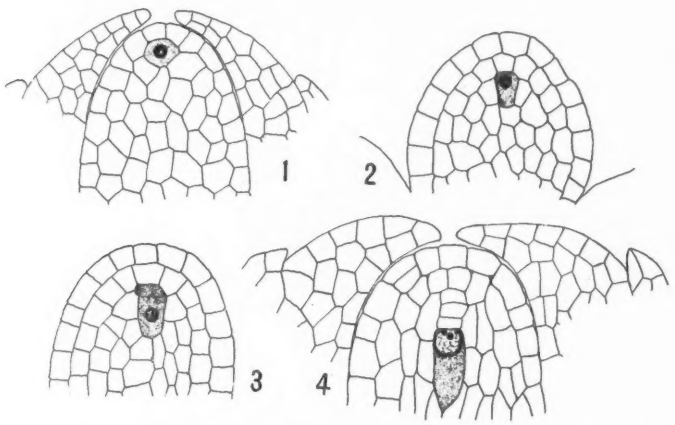


FIG. 1.—Ovule, showing archesporial cell. FIG. 2.—Same, after formation of two epidermal layers. FIG. 3.—First division of archesporial cell. FIG. 4.—The row of megaspores. All ×350.

nucellus and immediately under this investing layer is the archesporium (*fig. 1*). At this stage it differs from the surrounding cells neither in size nor in the nature of its contents, and can only be distinguished by its position.

As the nucellus grows, the cells of the outer layer divide, forming two layers outside the archesporium (*fig. 2*). The latter then divides transversely, cutting off a narrow tapetal cell (*fig. 3*). Up to this point the archesporial cell has undergone very little increase in size, but now the primary sporogenous cell begins to lengthen down-

ward rapidly, crowding the cells in the base of the nucellus, some of which break down. At the same time it undergoes two more transverse divisions, forming a row of three megaspores, the innermost of which functions (*fig. 4*).

The reduction division and the successive divisions up to the fertilization stage of the embryo sac proceed as usual (*fig. 5–7*). In the reduction division eight chromosomes were observed, and sixteen were counted in the vegetative cells. During the maturing of the embryo sac, it lengthens and becomes from one and a half to two times as

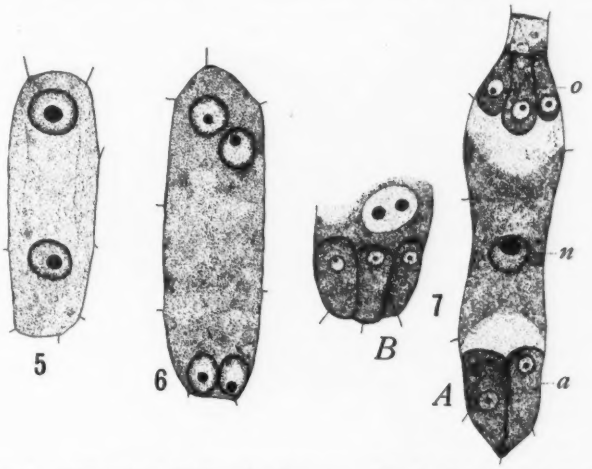


FIG. 5.—First nuclear division of embryo sac. FIG. 6.—Second nuclear division of embryo sac. FIG. 7.—*A*, mature embryo sac; *o*, oosphere; *n*, primary endosperm nucleus; *a*, antipodal cells; *B*, antipodal cells, and endosperm nucleus with two nucleoli. All ×900.

long as it was at the first division of the megaspore nucleus. The increase is all toward the chalaza, with very little lateral increase. In fact, as the sac elongates it frequently contracts in the middle.

Of the material examined early in March, very few ovules were found in which the division of the archesporial cell had taken place. In many of the ovaries examined about April first the embryo sac was in the fertilization stage.[3]

[3] I give the earliest date at which the plant develops to the stage of growth described. As a matter of fact, many plants are belated, and flower buds may sometimes be found in the middle of April still unopened; but this is exceptional.

FERTILIZATION

The process of fertilization takes place during April or early in May. It may follow quickly upon the completion of the division of the nuclei in the embryo sac, or the latter may remain for several weeks before being fertilized. All depends upon the accident of pollination. Spathyema is fertilized by the agency of insects, especially certain spiders which make a practice of spinning their webs inside the spathe and flies which are attracted by the rank odor of the plant.

The exact length of time required for the penetration of the pollen tube into the embryo sac was not determined. The stigmatic surface consists of a mass of loosely compacted cylindrical cells, thin-walled and not distinctly nucleated. Four vascular bundles run through the thick pyramidal style, corresponding to its four angles. The central portion of the style consists of a loose mass of thin-walled cells, through which the pollen tube readily forces its way to the upper end of the ovary. It then follows either the inner surface of the ovary or the outer surface of the integuments of the ovule until opposite the micropyle, through which it passes.

THE EMBRYO

After fertilization, the oospore by transverse divisions forms a proembryo of four cells. The terminal cell then divides longitudinally, followed by a second longitudinal division at right angles to the first. Each of the four terminal cells then divides transversely. The most advanced material examined during the second week in May showed the embryo in this stage of division. Beyond that point it has not as yet been possible to carry the investigation.

BLAIRSTOWN, N. J.

BRIEFER ARTICLES

PRELIMINARY NOTE ON CERATOZAMIA

With the aid of a grant made by the Botanical Society of America at the New Orleans meeting in December 1906, the writer has been able to secure material for a study of Ceratozamia in most phases of its life history. In September 1906 a trip was made to the barrancas of the Almolonga Valley near Xalapa, Mexico, where the plant is quite abundant. Photographs and field notes were secured and material was fixed for a detailed morphological and cytological study. Arrangements were also made for obtaining material at frequent intervals.

In habitat Ceratozamia differs decidedly from Dioon, for Dioon is in the open, exposed to blazing sunlight, while Ceratozamia is in densely shaded places. The difference in light will be appreciated from the fact that a photographic plate which would be well exposed for Dioon in one-fifth of a second would require three minutes exposure for Ceratozamia. On the whole, Dioon and the plants associated with it are xerophytic; while Ceratozamia, though not found in wet situations, is associated with a luxuriant vegetation.

Staminate cones which arrived in Chicago on March 10, 1906, shed their pollen within a week. Fertilization takes place more than a year after pollination. The first motile spermatozoids were observed June 9, 1906, and in the last week of June nearly every nucellus showed one or more pollen tubes in which spermatozoids were swimming. The spermatozoids were observed with diminishing frequency during the first two weeks in July. Swimming spermatozoids had previously been observed in Cycas by IKENO and in Zamia by WEBBER. Our observations add Dioon and Ceratozamia to the list. A section by LANG proves that the spermatozoid of Stangeria is also motile. Nothing is known of the pollen tube structures of the other four genera of cycads.

The ovulate cones disintegrate and free the seeds soon after fertilization, while the embryo is still of the same diameter as the filamentous suspensor. The seed has no resting period, but growth is continuous from fertilization to the leafy plant.—CHARLES J. CHAMBERLAIN, *The University of Chicago.*

CURRENT LITERATURE

BOOK REVIEWS

Progress of botany

In connection with the Vienna Congress, a series of papers describing recent progress in the various fields of botanical activity is being published under the editorship of Dr. J. P. LOTSY. The general title of the series is *Progressus rei botanicae*, and the first part, comprising four papers, has just appeared.[1]

ONTOGENY OF THE CELL.—Under this title STRASBURGER[2] gives an interesting and valuable history of the progress of cell studies since 1875. The various problems which in their turn have been most prominent in cytological investigation are treated with a breadth and grasp which would be impossible except for one who has contributed to the subject during its entire development. Particularly interesting is the treatment of problems like the centrosome, the origin of the spindle, double fertilization, etc., which have passed through the stage of dispute and polemic to comparative agreement. The treatment of the centrosome, which just now is perhaps the most investigated feature of the cell, is suggestive, the earlier problems, which are practically settled, being clearly marked off from the seemingly more difficult problems which are now occupying the attention of investigators. Text cuts are numerous and the bibliography is quite complete.—CHARLES J. CHAMBERLAIN.

PRESENT POSITION OF PALEOZOIC BOTANY.—SCOTT[3] has contributed a most timely account of the present status of our knowledge of paleozoic plants. The recent progress in this field has been most remarkable, and Professor SCOTT's connection with it has been conspicuous. Naturally the chief attention is given to the vascular plants, JEFFREY's division of them into Lycopsida and Pteropsida being used for convenience of presentation. Under the first division, Sphenophyllales, Equisetales, Psilotales, and Lycopodiales are presented; and under the second, Filicales, "Pteridospermeae," and "Gymnospermeae." Chief interest centers about the Filicales and pteridosperms. The question as to the existence of paleozoic ferns is answered decidedly in the affirmative; but with the statement that at present our knowledge of the group centers in the Botryopterideae, "which have now come to be of supreme interest in the geological history of vascular plants." The development of our knowledge of pteridosperms is

[1] Progressus rei botanicae. Herausgegeben von der Association Internationale des Botanistes. Redigiert von Dr. J. P. LOTSY. Erster Band. Erstes Heft. pp. 317. Jena: Gustav Fischer. 1907. *M.* 18.

[2] STRASBURGER, EDUARD, Die Ontogenie der Zelle seit 1875. Op. cit. 1:1–138.

[3] SCOTT, D. H., The present position of paleozoic botany. Op. cit. 1:139–217.

told briefly and clearly, and their separation from gymnosperms as a coordinate group is defended, the provisional diagnosis being as follows: "Male and female sporophylls little differentiated from the vegetative foliage; no cones formed. Anatomy of either stem, or leaf, or both, of a Filicinean type, as was also the habit." It is hard to see that such characters are more important that those which distinguish the acknowledged groups of gymnosperms, and of equal importance with those that distinguish gymnosperms from angiosperms. The relations of the pteridosperms to the Cycadophyta and to the Cordaitales are also discussed. The whole paper is an admirable résumé of our knowledge of the subject, and will serve to present it clearly to many botanists who have either no time to consult the numerous original papers or no access to them.

BIBLIOGRAPHY OF PALEOZOIC FOSSIL PLANTS.—The third paper is an exceedingly useful bibliography prepared by ARBER.[4] There is first a list of general monographs and textbooks, followed by a list of memoirs on special subjects, after which the titles are arranged stratigraphically, beginning with the Silurian. Under each geological period, beginning with the Devonian, the arrangement is by plant groups and by countries.

GEOGRAPHIC BOTANY.—The fourth paper is by FLAHAULT.[5] After a general historical and explanatory introduction, the subject is presented under the following captions: (1) Descriptive phytogeography (Floristics), (2) Physiological phytogeography (Ecology), (3) Ontogenetic phytogeography, and (4) Historical phytogeography. The second topic occupies the largest space, the principal subtopics being discussions of the ecological factors, temperature, water, light, soil, humus, and the various so-called climates. Ontogenetic phytogeography deals with the history of the distribution of plants, including the historical origin of groups, migrations, and the succession of floras. Under historical phytogeography the work of man in relation to floras is discussed, including such subjects as the sources of rivers, mountain forests, dunes, etc. The paper brings together in an organized form the study of vegetation from the various points of view that have been often lumped under the general and very indefinite title Ecology. —J. M. C.

A South African textbook in botany

This is not the first elementary textbook written for use in the South African schools, but it is perhaps the first to give so much attention to the ecological point of view.[6] In this respect it is to be compared with the elementary text-

[4] ARBER, E. A., NEWELL, Bibliography of literature on palaeozoic fossil plants, including some of the more important memoirs published between 1870–1905. Op. cit. 1:218–242.

[5] FLAHAULT, CH., Les progrs de la géographie botanique depuis 1884, son état actuel, ses problèmes. Op. cit. 1:243–317.

[6] STONEMAN, BERTHA, Plants and their ways in South Africa. 8vo. pp. ix+283. London: Longmans, Green, & Co. 1906. $1.10.

books of COULTER or BERGEN. It is refreshing to miss the old familiar cuts that have done service for so long. Not only do the photographic reproductions represent South African scenes, but even the diagrams and cuts are made from South African plants, and for the most part especially for this book. The first chapters deal with seeds and germination, growth and duration of the vegetative organs, and various fundamental physiological topics. The chapter on plant defenses is illustrated by some of the remarkable desert xerophytes of the region, such as Crassula and Mesembryanthemum. Then follow chapters on vegetative reproduction, climbing plants, and migration. After a rather full consideration of flowers and fruits, there is a simple but useful key to the more conspicuous plants of the region. The old-fashioned order of BENTHAM and HOOKER is used in the classification, but this is probably justified by the fact that South African systematic works have followed the BENTHAM and HOOKER scheme. The book should do its part in making botany in the schools a more living and attractive subject.—HENRY C. COWLES.

MINOR NOTICES

Spontaneous heating.—The "heating" of hay and compost heaps has been an interesting biological phenomenon as to whose causes many were content to reason, but few to experiment. MIEHE recently published a scientific summary of his work, which is now followed by a booklet, addressed to a wider audience.[7] It embodies his own investigations for the past two years, to which are added the facts already known, so as to make a complete discussion of this subject, which is of both biological and practical interest.

The topics treated are: The course and maximum of the rise of temperature; chemical changes in the heating of compacted hay with exclusion of oxgyen; cause of heating; culture substrata and researches with pure cultures; description of the nine most important organisms found in hay; self-sterilization by heating; the conditions of existence of thermophilous organisms in nature; heating plant materials as brood-beds for pathogenic organisms; relation of the fermentation of tobacco to the heating of hay; respiration and warmth; heating and spontaneous combustion, present and past.

The book will be particularly useful to men in experiment stations who are concerned with this problem, as it forms an excellent summary of present knowledge. It is handicapped by lacking an index, though it has an exhaustive bibliography.—C. R. B.

The origin of species and varieties by mutation.—It is an encouraging sign when such a scientific treatise as Professor DEVRIES' *Species and varieties* comes to a second edition within one year.[8] Probably no work on evolution written in

[7] MIEHE, HUGO, Die Selbsterhitzung des Heus. 8vo. vi+127. *figs.* 11. Jena: G. Fischer. *M*3.50.

[8] DEVRIES, HUGO, Species and varieties; their origin by mutation. Edited by D. T. MACDOUGAL. Second edition. 8vo., pp. xviii+847. Chicago: The Open Court Publishing Co. 1906.

English has had such a great sale, at least for many years. Very few changes are to be seen in the new edition. The frontispiece shows Professor DEVRIES at the Desert Laboratory in somewhat informal garb. The few errors of the first edition have been corrected, and some alterations have been made for the sake of clearness. The most important new feature is an explanatory note on variations in *Oenothera biennis*. It is to be hoped that the sale of this second edition will also be great enough to necessitate the publication of yet another edition. In this way it will be possible for the general public to keep conversant with the rapid advance of our knowledge concerning mutation.—H. C. COWLES.

NOTES FOR STUDENTS

Fixation of atmospheric nitrogen.—In agricultural practice this is a most important matter; witness the many efforts to secure it in leguminous crops by inoculating the soil. Two recent elaborate papers concern themselves with the organisms which are supposed to do this in the soil, and from these studies it is clear that our knowledge of this process and its conditions rests upon most insecure foundations.

WARMBOLD, reviewing the literature relating to the alteration of the N-content of uncultivated soils, finds such a contradiction between the data, particularly those of BERTHELOT, who found N-fixation active in summer but nil in winter, and KOCH, who found it notable in winter, that he endeavored to determine the conditions for the proceess in nature, especially in relation to temperature, water content of soil, aeration (dependent on porosity of soil), and the amount and character of organic substances present.[9] Having carefully considered the sources of error in the methods of determining N in the soil, he believes his analytic results may be relied on; and with a warning against too wide generalization from his data he concludes: (1) Well-aerated soils may be enriched in N without the intermediation of organisms, this having occurred in two series of experiments, while in three others under apparently like conditions it did not take place. (2) Temperature exercises no obvious influence, nor does water content, provided the soil is in thin layers and well aerated. When, however, it is in masses and contains less than 20 per cent. water, accumulation of N is either small or a minus quantity; at 10 per cent. there may be marked loss of N. This difference in behavior depends on a difference in the N-compound in question. With 3 per cent. water or less, the loss in N is due not to denitrification by organisms, but is purely chemical. In sterilized soil 3–20 per cent. of water has no effect; at 30 per cent. N diminishes.[10] (3) Aeration had no discernible effect in small masses of soil, but it was advantageous in larger quantities containing 15 per cent.

[9] WARMBOLD, H., Untersuchungen über die Biologie stickstoffbindender Bakterein. Ein Beitrag zur Kenntnis der Veränderungen in Stickstoffgehalte des unbebauten Ackerbodens. Landw. Jahrb. **35**:1–123. 1906.

[10] But *cf.* as to this point SCHULZE on the effects of sterilizing soils. Abstract in BOT. GAZETTE **42**:502. 1906.

water. (4) Artificially prepared humus neither affected the N-fixation favorably, nor could the bacteria use it as a source of food. (5) The N-fixing power of supplies of Azotobacter derived from pure cultures and grown in identical conditions was extraordinarily different at optimal temperatures as well as at low and higher points. Some light may be cast on this by the following.

In an elaborate examination of the behavior of Azotobacter[11] THIELE believes that he has established incontestably that this organism is capable of accumulating N in the laboratory; but he is quite uncertain whether this power belongs specifically to it, as for example alcohol production does to yeast. It is not impossible that N-starvation in the artificial culture or the stimulation by abundance of organic matter awakens an inherent capacity of Azotobacter to fix N, which ordinarily slumbers. The growth of Azotobacter in artificial cultures is neither decisive nor typical. Its mode of action in the soil is still entirely unknown and is likely to remain so, in spite of theories, until there have been devised more exact methods of investigating the extremely minute variations of the N in soils. In view of all this uncertainty, THIELE deprecates giving agriculturists any advice which would lead them to attempt to replace Chili saltpeter by a bacterial "fertilizer."—C. R. B.

Burbank's work.—An interesting and illuminating account of the breeding experiments of LUTHER BURBANK is contributed by DEVRIES to the *Biologisches Centralblatt.*[12] It is the first statement we have seen addressed to scientific men by a man competent to appreciate both the practical and scientific aspects of BURBANK'S work.—C. R. B.

Anti-enzyme.—With the aid of BERTEL, CZAPEK[13] has now elaborated the results of ten years of research devoted to developing a chemical test for tropistic sensation. Until 1897, when the author published his initial paper of this investigation, we had no way of knowing that an organ had perceived a stimulus unless it manifested response in the form of a motor reaction. In fact it was assumed that perception had not occurred unless such a motor reaction followed. FITTING, in his work on tendrils, was able by indirect methods to show that an organ may perceive a stimulus and still be incapable of executing a motor reaction. In animal irritability it has long been believed that sensations involve alterations in the metabolism of the organ. To CZAPEK belongs the honor of a fruitful pioneer research. Root tips contain tyrosin, an unstable derivative of proteids, continually yielding oxidation derivatives. The most prominent of the latter is homogentisinic acid. This research has shown that this acid is present in unstim-

[11] THIELE, R., Die Verarbeitung des atmosphräischen Stickstoffs durch Mikroorganismen. Landw. Versuchs-Stat. **63**:161–238. 1906.

[12] DE VRIES, HUGO, Die Neuzüchtungen Luther Burbank's. Biol. Centralb. **26**:609–621. 1906.

[13] CZAPEK, FRIEDRICH, unter Mitwirkung von RUDOLPH BERTEL, Oxydative Stoffwechselvorgänge bei pflanzlichen Reizreaktionen. (Zwei Abhandlungen.) Jahrb. Wiss. Bot. **43**:361–467. 1906.

ulated tips in about the quantity of 16 per cent., while in stimulated tips it is present to the extent of about 20 per cent. Such figures can be obtained with considerable accuracy by an improved application of the silver reduction test. The increased reduction of an ammoniacal solution of silver nitrate following stimulation (geotropic or phototropic) can be positively shown to be due to the increased quantity of homogentisinic acid. The oxidation of this acid is accomplished in ordinary metabolism by an enzyme, which because of its activity with phenol is called phenolase. The normal activity of this enzyme prevents accumulation of homogentisinic acid above *c.* 16 per cent. In consequence of stimulation, however, there arises a substance (*de novo* so far as our knowledge now goes) which inhibits the activity of the enzyme, and thus the accumulation of homogentisinic acid up to 20 per cent. is indirectly permitted This new substance is called anti-enzyme. For different organs of the same individual and for different individuals of close systematic relationship this anti-enzyme is identical. With decreasing relationship, however, the actual substance of the anti-enzyme seems to differ, though the function remains constant. Chloroform narcosis, suffocation, antipyrin, acids, alkalies, various traumatic influences, mechanical interference with growth, and universal illumination all gave negative results for an anti-enzyme. Thus the author believes that this increased reduction of silver following stimulation is a safe and reliable test for tropistic sensation.—RAYMOND H. POND.

Behavior of liverworts in darkness.—NĚMEC has been experimenting with certain bryophytes which will grow in darkness and are not geotropic, to see what light they can throw on the mode of perception of geotropic stimulus, the biological significance of etiolation, and spontaneous nutations.[14] He finds different species behave very differently in darkness; some do not grow, others make a little growth but show no etiolation, while yet others grow long and vigorously and become markedly etiolated. Of the last, most are geotropic and grow erect or obliquely upward. But *Lejeunea bidentata* and *L. serpyllifolia* are ageotropic, having no starch or other statoliths. In darkness their shoots are completely unoriented, being at first hyponastic and then nutating irregularly. The same disorientation is observable in the sporogone of *Aneura pinguis*, but the vegetative shoots are geotropic and contain abundant statolith starch. The sporogone of *Pellia calycina* behaves similarly, but is slightly geotropic at first, losing this during elongation of the seta, which contains still some starch diffused between cell wall and vacuole and of slight mobility. The sporogone of *Pellia epiphylla*, on the contrary, is strongly geotropic and has abundant mobile starch. The sporogones of the three last named are strongly positively phototropic, and the reaction is in no way connected with the capsule. Also the upper part of the seta may be cut off, neither wound shock (which is transient) nor removal interfering with the reaction. The vegetative shoots of *Pellia calycina* grow well (plagi-

[14] NĚMEC, B., Die Wachsthumsrichtungen einiger Lebermoose. Flora **96**:409–450. *figs.* 9. 1906.

otropically) in darkness, and give a very characteristic geotropic reaction, having abundant statolith starch. *P. epiphylla* generally does not grow in darkness. Neither these two nor *Lophocolea bidentata* are geotropic in light. The behavior in darkness of *P. epiphylla*, the two species of Lophocolea, and the sporogone of Aneura must be considered as purposeless.

The researches appear to strengthen the statolith theory of geotropic perception.—C. R. B.

Photosynthesis.—USHER and PRIESTLY, having shown in a previous paper[15] that CO_2 may be decomposed in plants independently of enzymic or "vital" activity provided the products (H_2O_2 and H·COH) are removed, have now devised an arrangement by which photolysis of CO_2 can be produced *in vitro*.[16] By covering gelatin plates, or even water, with a very thin uniform layer of chlorophyll deposited from solution, and placing the glass plate or the shallow dish of water carrying the film in a vessel with CO_2 and illuminating it, they found formaldehyde produced and were able to recover identifiable quantities. By making up the gelatin with an aqueous solution of a catalase, the hydrogen peroxid also produced was decomposed with the evolution of O_2, 2^{cc} being obtained in one case. The chlorophyll was gradually bleached, in harmony with the view that it acts as a sensitizer and is destroyed in the process of photolysis. Synthesis of H·COH into carbohydrate was found in the earlier paper to be dependent on the healthy condition of the protoplast, and feeding experiments have already shown that H·COH when supplied in very dilute form can be condensed by green plants if illuminated. The authors painted the white petals of *Saxifraga Wallacei* with chlorophyll, and floated them on water charged with CO_2 in light. In the course of a day they were found to contain starch. Thus they secured photosynthesis by a different (though inefficient) arrangement of the mechanism of a green leaf.

Further study was made of photolysis of CO_2 in the presence of uranium salts. They have direct evidence of the production of formic acid (which also is produced under some conditions in the plant as an intermediate product in the reduction of CO_2), but they were unable to isolate and identify formaldehyde.

These papers record a most important step in solving the problems of photosynthesis.—C. R. B.

Vascular anatomy of cycads.—Two years ago MATTE published his thesis on the vascular anatomy of the cycads,[17] in which he presented in great detail, with copious illustrations, the vascular anatomy of the leaves and flowers in representative species of all the nine genera; and of the seedlings of *Dioon edule, Cycas*

[15] USHER, F. L., and PRIESTLY, J. H., A study of the mechanism of carbon assimilation in green plants. Proc. Roy. Soc. London B. **77**:369–376. 1905.

[16] ——The mechanism of carbon assimilation in green plants: the photolytic decomposition of carbon dioxid *in vitro*. *Idem* **78**:318–327. 1906.

[17] MATTE, H., Recherches sur l'appareil libero-ligneux des Cycadées. pp. 233. *pls. 16. figs. 264.* 1904.

siamensis, and *Encephalartos Barteri*. He lays special stress on the strands of the foliar organs, his unit of primary importance in relation to phylogeny being what he calls the *meriphyte*, the vascular axis of the stem being of secondary importance. Among other conclusions in reference to the strands of the leaves, he states (1) that the course of the vascular strands sometimes presents great complexities, and (2) that the classic Ω of the foliar arc, although usual, is not general, referring particularly to Bowenia. A later paper[18] deals with the structure of the meriphyte of *Bowenia spectabilis*, and attempts to prove that the complex arrangement of the foliar strands even in this form can be reduced to the typical Ω.—R. THIESSEN.

In an earlier paper MATTE[19] noted in certain ovulate sporophylls of Ceratozamia the occurrence of three or more vascular strands near the abaxial surface of the sporophyll, in addition to the row of strands that usually appears in transverse section. These accessory strands showed reversed orientation, the xylem being on the abaxial side; and MATTE regards this as an evidence of the union of two sporophylls along their edges.—CHARLES J. CHAMBERLAIN.

Problems of nutrition.—ARTARI published in 1904 an account of the influence of the different concentrations of glucose and saccharose upon the development of *Stichococcus bacillaris* and certain lichen-algae. This he now supplements[20] by investigating the effect of different concentrations of each component of the nutritive solutions upon the amount and energy of multiplication in *Stichococcus Chlorella* (a new physiological species) and the algae from *Xanthoria parietina*.

The concentration of NH_4NO_3, which was the source of N, has no effect on Chlorella below 0.5 per cent., and on Stichococcus below 1 per cent.; but higher concentrations retard development, and suggest that the salt acts also in another way than by its osmotic pressure. The nutritive value of various sources of N varies according as glucose is present or absent. Glucose itself exercises an observable effect even at 0.005 per cent., and acts best between 0.5 and 2 per cent. The limiting concentration of monosaccharides, varying more or less with different algae, seems to be related to molecular weight, since it is about equal in monosaccharides (glucose, galactose, and fructose), and approximately double this limit in disaccharides (saccharose and lactose), which also agree together. Many other details, not easily related, are also recorded. A few observations upon the effect of concentration upon the form and size of cells are given incidentally; the author seems not to know of LIVINGSTON'S papers on this subject. —C. R. B.

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[18] MATTE, H., Compléments à la structure mériphytaire du *Bowenia spectabilis*. Compt. Rend. Acad. Sci. Paris, 409–416. 1905.

[19] MATTE, H., Une anomalie de structure dans l'écaille ovulifère de *Ceratozamia mexicana*. Bull. Soc. Linn. Normandie V. **7**:52–54. 1903.

[20] ARTARI, A., Der Einfluss der Konzentrationen der Nährlösungen auf die Entwicklung einiger grüner Algen. II. Jahrb. Wiss. Bot. **43**:177–214. 1906.

Tetrads of a Drosera hybrid.—ROSENBERG[21] states that in the genus Drosera the pollen cells remain united in tetrads at maturity, even after pollination. In the hybrid *D. rotundifolia*×*D. longifolia*, in which he previously studied the earlier stages of pollen development,[22] he now describes the mature stage after the reduction division. Instead of four pollen cells in such a tetrad, he finds occasionally in the hybrid additional small pollen grains, which arise from extra nuclei formed by chromosomes left behind in the cytoplasm during the reduction divisions. The tetrad groups of the parent species are distinguishable by the size and shape of their individual grains, those of *D. rotundifolia* being smaller and rounded at the outer edge, those of *D. longifolia* larger and flattened. In most of the tetrads of the hybrid containing only four pollen cells, all the grains have the size and shape of *D. longifolia;* but occasionally two cells have the characters of one parent and two those of the other. He concludes that in the distribution of chromosomes in the reduction divisions, chromosomes from both parents usually enter all the daughter nuclei, but that occasionally in the first division the chromosomes of *D. rotundifolia* are more or less completely segregated from those of *D. longifolia*, two of the pollen cells of such a tetrad having the characters of the pollen of each parent.—R. R. GATES.

Items of taxonomic interest.—S. H. BURNHAM (Torreya **6**:235. 1906) has described a new Monotropsis from the Blue Ridge Mountains.—W. H. BLANCHARD (*idem* 236) has described a new Rubus (dwarf blackberry) from Vermont.—B. F. BUSH (Rept. Mo. Bot. Garden 119–125. 1906) has described new Texan species under Tracyacanthus, Allium, Psoralea (2), Tragia, Xanthium Antennaria, and Silphium.—A. S. HITCHCOCK (Rhodora **8**:205–212. 1906), among notes on grasses of the N. E. United States, describes new species in Panicum (4) and Glyceria.—W. H. BLANCHARD (*idem* 212–218) describes 2 new species of Rubus (blackberries) from Maine.—A. BERGER (Notizblatt **4**:250. 1906) has described a new Agave from Mexico.—H. H. HAINES (Jour. Linn. Soc. **37**:407–409. 1906) has described 2 new species of Populus from India.—O. STAPF (*idem* 495–532), among descriptions of numerous new species from the Dawe's collection from Uganda, publishes the new genus *Blasamocitrus* (Rutaceae).—J. HUBER (Boletim Mus. Goeldi **4**:510–619. 1906), in his sixth contribution to a flora of the Amazons, describes *Browneopsis* as a new genus of Leguminosae (Caesalpinioideae). J. C. ARTHUR (Journal of Mycology **13**:28–32. 1907) has published *Polioma*, *Spirechina*, *Prospodium*, and *Nephlyctis* as new genera of Uredinales.—PH. VAN TEIGHEM (Ann. Sci. Nat. Bot. IX. **4**:223–260. 1906) has established the new dicotyledonous family Agialidaceae, to include certain spiny trees and shrubs referred usually to Balanites (Simarubaceae), the

[21] ROSENBERG, O., Erblichkeitsgesetze und Chromosomen. Botaniska Studier, tillägnade F. R. KJELLMAN **1906**:237–243. *figs.* 5.

[22] ——, Das Verhalten der Chromosomen einer hybriden Pflanze. Ber. Deutsch. Bot. Gesells. **21**:110. 1903.—Uber die Tetradentheilung eines Droserabastardes. Ber. Deutsch. Bot. Gesells. **22**:47. 1904.

family containing the genera Balanites, Agialida, and *Agiella* (new); the same author (*idem* 261–271) also breaks up the Boraginaceae into 4 families, Boraginaceae, Ehretiaceae, Cordiaceae, and Heliotropiaceae.—C. V. PIPER (Contrib. U. S. Nat. Herb. **10**:1–48. *pls. 1–15.* 1906), in a revision of N. Am. species of Festuca, recognizes 34 and describes 3 as new.—A. A. HELLER (Muhlenbergia **2**:177–256. 1906), in an account of botanical exploration in California during 1906, in which numerous species are noted and nomenclatural changes made, describes new species in Taxicoscordion, Eriogonum (2), Mirabilis, Lupinus (4), Hesperastragalus, Anogra, Chylisma, Phlox, Phacelia, Conanthus, Cryptanthe (2), Amsinckia, Pentstemon (2), and Orthocarpus.—PHILIP DOWELL (Bull. Torr. Bot. Club **33**:547–556. *pls. 18–22.* 1906), in a revision of N. Am. species of Calceolaria, recognizes 16 and describes 6 as new.—J. M. C.

Formative influence of light.—PEIRCE has extended his studies on irritability in plants from algae to liverworts and ferns, recording the effect of light upon germination and early growth in *Anthoceros fusiformis, A. Pearsoni, Fimbriaria californica,* and *Gymnogramme triangularis,* and its effect on form in later stages of growth.[23] He finds that germination is dependent upon light of undetermined intensity, and that direction of growth and of successive cell division is determined by the direction of light. The direction of light also profoundly modifies the form of the thalli of the liverworts. This was most marked in Anthoceros. On a clinostat they tend to become solid, erect, and cylindrical, conic or vasiform, with radial structure instead of dorsiventral. The author holds that his early results support the hypothesis that, aside from what is actually transmitted from parents to offspring, likeness is due to likeness or identity of the physical environment, and that these factors are as essential determinants as the substance transmitted. Unfortunately PEIRCE'S experiments were interrupted by the earthquake of April 16, so that conclusive tests have not been made in all cases. The work will be continued.—C. R. B.

Assimilation of organic acids by algae.—An interesting contribution to our knowledge of the power of assimilation of carbon compounds by autotrophic plants has been made by TRÉBOUX.[24] This investigator experimented with some forty species of the lower algae, testing their power of assimilating various organic acids, which were given in the form of potassium or ammonium salts. Cultures were kept in absolute darkness. It was found that about one-half of the species flourished under the cultural conditions with an organic acid as the only source of carbon; and that of all the acids used acetic acid was most readily assimilated, while acids with larger carbon chains were assimilated in only a few cases. In some cases aminoacids were used with accompanying escape of ammonia. This

[23] PEIRCE, G. J., Studies of irritability in plants. Annals of Botany **20**:449–465. *pl. 35.* 1906.

[24] TRÉBOUX, O., Organische Säuren als Kohlenstoffquelle bei Algen. Ber. Deutsch. Bot. Gesells. **23**:432–441. 1906.

work contributes additional evidence showing that the line between autotrophic and heterotrophic nutrition is not so sharply drawn as is generally supposed. The power of the lower algae to use carbon compounds and especially the lower acids explains the abundant growth of those forms where decaying organic matter is present, as in contaminated waters.—H. HASSELBRING.

Rhizomorphic root-rot of vine.—MAGNUS[25] describes a rhizomorphic root-rot of the vine which causes the death of numerous plants in the vineyards of certain regions of Europe. The rhizomorphs are white strands which may attain a diameter of 2^{mm}. The strands lack a well-developed cortex, thereby differing from the characteristic strands of *Armillaria mellea*. The fungus is found to be abundant on the posts used as supports for the vines. From the posts the strands spread to the young roots of the vine, entwining and killing them. Although this disease with the accompanying rhizomorphs had been known for several years, it was not until the present year that the connection of the rhizomorphs with one of the Hymenomycetes was established by finding numerous sporophores of *Collybia platyphylla* growing from the strands. As this fungus is very common in America, it is not unlikely that it may be connected with one of the numerous rhizomorphic root-rot diseases infecting our fruit trees.—H. HASSELBRING.

Segregates of Rhus glabra.—GREENE[26] has studied the forms referred to *Rhus glabra* throughout its reputed range. He remarks that "there is no one species of tree or shrub of any continent that really holds the geographic range which the books and lists ascribe to *Rhus glabra*;" and points out the exceedingly diverse areas it is said to occupy in its continental distribution. Accordingly he has begun its segregation on the basis of such herbarium material as is available, recognizing the fact that this is probably a very scanty showing of the real situation, for "no special call has been made for collecting these shrubs from different regions." The form chosen to stand for the original *R. glabra* L. is one ranging from eastern Virginia and southern Maryland through southern Pennsylvania to Connecticut. Outside of that region botanists will have to refer their reputed forms of *R. glabra* to other species. This initial work of segregation has resulted in 29 species, 24 of which are new.—J. M. C.

Transpiration.—ARESCHOUG maintains the correctness of his view that the palisade tissue, when well developed and compact, reduces transpiration,[27] explaining away certain apparently contradictory experiments of HESSELMAN and others.

It seems to be about time to dismiss the idea of transpiration as a function,

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[25] MAGNUS, P., Ueber eine Erkrankung des Weinstockes. Ber. Deutsch. Bot. Gesells. **24**:402–406. 1906.

[26] GREENE, EDWARD L., A study of *Rhus glabra*. Proc. Wash. Acad. Sci. **8**: 167–196. 1906.

[27] ARESCHOUG, F. W. C., Ueber die Bedeutung des Palisadenparenchyms für die Transpiration der Blätter. Flora **96**:329–336. 1906.

of which leaves are the organs, and to consider it as a merely unavoidable evaporation whose amount depends upon physical factors that need to be exactly evaluated. What would be thought of an engineer who attempted to compare the performance of two boilers of different construction if he knew nothing of the heat units applied? Yet most of the so-called "comparative" studies of transpiration take no account of the fundamental energetics involved, assuming that when leaves are placed "under the same conditions" they have a like amount of energy for transpiration!—C. R. B.

Correlation and leaf size.—A. J. EWART has published a short note dealing with correlation and leaf size.[28] LINDEMUTH has shown that adult leaves of Begonia and Iresine increase in size when allowed to root in the soil. EWART conducted experiments on *Tilia europaea*, and found that adult leaves do not increase in size, even when most of the leaves are removed. However, a partial defoliation of young shoots causes a development of unusually large leaves, and the increase in size beyond the normal is due to an increase in the number of cells and not to an increase in their size, as supposed by LINDEMUTH. That increased size is due to an increased number of cells in such cases is not the common view.—H. C. COWLES.

Presynapsis and synapsis.—Presynaptic and synaptic stages in the first division of the embryo sac mother cell of Adoxa are interpreted by LAGERBERG as follows.[29] Before synapsis the chromatin granules collect into groups which are often in pairs, apparently less numerous than the mature chromosomes. The chromatic substance of the groups becomes distributed along parallel linin threads, so that at the beginning of synapsis there are two parallel threads which fuse as synapsis proceeds. The single thread which is thus formed is the thickest which appears at any time in this nucleus. Accordingly, the fusion of the male and female elements of the nucleus takes place during synapsis.—CHARLES J. CHAMBERLAIN.

Embryology of Capsella.—The late Mrs. Mabel Schaffner[30] left an incomplete paper on the embryology of Capsella, which her husband, Professor JOHN H. SCHAFFNER, has published. It is a detailed study of the development of the embryo of what is perhaps the most frequently used dicotyledon in teaching. Aside from the completeness of the series, the striking feature of the plates is that the whole series is drawn to the same scale, representing to the eye the actual increase of the embryo in size at each stage of its development.—J. M. C.

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[28] EWART, A. J., The influence of correlation upon the size of leaves. Annals of Botany **20**:79–82. 1906.

[29] LAGERBERG, TORSTEN, Ueber die präsynaptische und synaptische Entwickelung der Kerne in der Embryosackmutterzellen von *Adaxa moschatellina*. Botaniska Studier, tillägnade F. R. KJELLMAN. **1906**:80–88.

[30] SCHAFFNER, MABEL, The embryology of the shepherd's purse. Ohio Nat. **7**:1–8. *pls. 1–3*. 1906.

Leaf structure.—Most botanists are familiar with the contributions of Dr. COCKAYNE to our knowledge of the flora of New Zealand and the adjacent islands. On his trip to the southern islands several years ago, he brought back a number of living plants which are now growing in the rockery of Canterbury College. These plants have been made the subject of an anatomical investigation by Miss HERRIOTT.[31] In spite of the uniform climate of the southern islands, many if not most of the plants reveal xerophytic structures. The author cites two conditions in these island regions which may be regarded as xerophytic. One is the frequence and violence of the wind, and the other is the peaty soil.—H. C. COWLES.

Forestry.—Two recent bulletins of the Bureau of Forestry will be of some interest to ecological workers. One of the bulletins[32] describes the valley and ridge forests of Kansas and Nebraska and their economic possibilities. The natural extension of both types of forest is discussed, but this subject will be well known to all through work of Professor C. E. BESSEY. The other bulletin[33] is almost wholly of economic interest, but ecologists will find some notes on the natural reproduction of the white pine in the old fields of New England.—H. C. COWLES.

Hygroscopic mechanisms.—STEINBRINCK, who has given particular attention to hydrophysics, summarizes for non-technical readers the mechanisms of shrinkage and cohesion in plants.[34] It is these mechanisms which are concerned chiefly in the rupture of capsules and sporangia. The current conceptions as to the rupture of the latter particularly need correction, and the article is commended to students. In his *Literaturübersicht* he has indeed overlooked American literature on this subject.—C. R. B.

Biology of Dunaliella.—In 1905 TEODORESCO described[35] Dunaliella as a new genus of Volvocaceae, giving an account of its structure and life history. In a second paper[36] he has begun an account of certain "biological observations." In this first instalment these observations deal with changes in the form of the body, cell structure, cell division, sexual reproduction, and the resting condition.—J. M. C.

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[31] HERRIOTT, E. M., On the leaf structure of some plants from the southern islands of New Zealand. Trans. N. Z. Inst. **38**:377–422. 1906.

[32] KELLOGG, R. S., Forest belts of western Kansas and Nebraska. U. S. Dept. Agric., Forest Service, Bull. 66. 1905.

[33] SPRING, S. N., The natural replacement of white pine on old fields in New England. U. S. Dept. Agric., Bureau of Forestry, Bull. 63. 1905.

[34] STEINBRINCK, C., Ueber Schrumfungs- und Kohäsions-mechanismen von Pflanzen. Biol. Centralbl. **26**:657–677, 721–744. *figs. 28.* 1905.

[35] Beih. Bot. Centralb. **18**:215–232. 1905.

[36] TEODORESCO, E. C., Observations morphologiques et biologiques sur le genre Dunaliella. Rev. Gén. Botanique **18**:353–371. *pls. 2.* 1906.

Purple bacteria.—MOLISCH describes two new genera of purple bacteria isolated in the laboratory from jars of sea water, sea grass, and decaying crabs or star-fish.[37] For these forms he finds it necessary to add a new sub-family to WINOGRADSKY'S and MIGULA'S classification. This is sub-family VI. RHODOCAPSACEAE (cells free, not capable of swarming throughout life). Under this he places his two new forms, *Rhodocapsa suspensa* (cells sometimes capable of swarming, rods or threads with gelatinous capsule), and *Rhodothece pendens* (power of swarming not as yet observed, cells round, with gelatinous capsule). —MARY HEFFERAN.

Origin of the pollen tube.—According to WETTSTEIN,[38] the rhizoidal tubes of the cycads and Ginkgo are to be regarded as completely homologous with the pollen tubes of the higher gymnosperms and the angiosperms. The writer then attempts to show that the rhizoidal tube of the cycads and Ginkgo is homologous with the vegetative end cell of the heterosporous pteridophytes. The rudimentary or rhizoidal cell of the heterosporous pteridophytes does not lead to the rhizoidal tube of the cycads and Ginkgo, and is still represented in these lower gymnosperms by a prothallial cell. Chalazogamy is regarded not as a primitive condition, but merely as an interesting special case. The paper is a preliminary statement without figures.—CHARLES J. CHAMBERLAIN.

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[37] MOLISCH, HANS, Zwei neue Purpurbakterien mit Schwebekörperchen. Bot. Zeit. **64**:223–232. *pl. 8.* 1906.

[38] WETTSTEIN, R. VON, Der Ursprung des Pollenschlauches. Naturw. Rundschau **21**:1–2. 1906.

NEWS

DR. F. CAVARA has been appointed director of the Botanical Garden at Naples.

DR. LUDWIG DIELS, University of Berlin, is delivering a series of lectures at the University of Marburg.

DR. B. LONGO, lately of the University of Rome, has been appointed professor of botany at the University of Siena.

DR. ARTHUR HOLLICK has been appointed by the mayor a member of the Board of Education of Greater New York.

THE AMERICAN ASSOCIATION for the Advancement of Science will meet at the University of Chicago during the holiday week of 1907.

DR. J. B. FARMER has been appointed president of the section of botany of the British Association, which holds its next meeting at Leicester.

Dr. D. H. SCOTT has resigned his position as honorary keeper of the Jodrell Laboratory at Kew, a position which he has held since 1892.

PROFESSOR C. E. BESSEY, University of Nebraska, has been elected vice-president of Section G of the American Association for the coming year.

MISS CLARA E. CUMMINGS, professor of cryptogamic botany in Wellesley College, died on December 28. She had served Wellesley College since 1878.

A. G. TANSLEY, assistant professsor of botany, University College, London, and editor of the *New Phylologist*, has been appointed lecturer in botany to succeed Professor A. C. SEWARD.

MISS LILIAN M. WILCOCKSON, of the University of Minnesota, is arranging a "science and recreation camp" on the shore of Vermilion Lake, Minnesota, to begin about the middle of July. Work and play are offered *ad libitum*.

ABOUT HALF THE COLLECTIONS have been transferred from the old building in Berlin to the new Museum building of the Royal Botanic Garden at Dahlem. It is expected that the transfer will be complete by spring and the building then fully occupied.

THE HERBARIUM of the late WILLIAM MITTEN of England, one of the most important collections for American bryology, has been purchased by the New York Botanical Garden. We congratulate the director upon his enterprise and success in securing this valuable collection.

AT THE New York meeting of the Botanical Society of America the following officers were elected for the ensuing year: President, GEORGE F. ATKINSON, Cornell University; Vice-president, N. L. BRITTON, New York Botanical Garden; Secretary, D. S. JOHNSON, Johns Hopkins University; Treasurer, ARTHUR HOLLICK, Columbia University.